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BLOOD PRESSURE STUDIES IN SMALL ANIMALS[1]

R. A. WOODBURY AND W. F. HAMILTON

From the Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta, Georgia

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The mean blood pressures of animals as small as the rat and jackdaw have been measured with mercury manometers (1, 2, 3). The pressures were as follows: rat 70 to 100, guinea pig 75 to 90, rabbit 100, dog 120, and horse 155 mm. Hg. Birds are credited with higher pressures: jackdaw 119, pigeon 130, crow 150, buzzard 170, and turkey 190. Cold blooded animals averaged between 30 and 80 mm. Hg.

The object of this paper is to present a method, which can be used in experimental studies for recording the systolic and the diastolic pressures in small unanesthetized, uninjured animals, and to give some of the results and conclusions from our work. These include topics such as pulse contour, duration of systole and diastole, and the rate of descent of arterial pressure during diastole with its relationship to the volume elasticity coefficient of arteries.

Our animals did not suffer from blood loss and injected anticoagulant. These are factors which reduce blood pressure in small animals to a greater extent than in large animals.

Recent indirect methods such as those of Griffith et al. (4) and Bonsmann (5) have not been checked against systolic and diastolic pressures as determined directly by adequate manometers and are definitely lower than those for rats reported by Leiter (6) who used a mercury manometer and a cannula.

Our results indicate that small animals, contrary to general belief, have pressures similar to larger animals of the same class.

APPARATUS. Registration of the pressure pulses in small warm blooded animals requires an apparatus of high natural frequency even when equipped

[1] A portion of this work was done while visiting the Physiological Laboratories at the University of Wisconsin.

with a very small cannula. The optical manometer of Hamilton, Brewer and Brotman (7, 8) was used in making these studies.

The ventricular records were taken with 26 G. needles as cannulae, while the arterial records were taken with glass cannulae with tips as small as one-third the diameter of 26 G. needles and constructed with Frank's (9) principles in mind. These were made with a very abrupt taper near

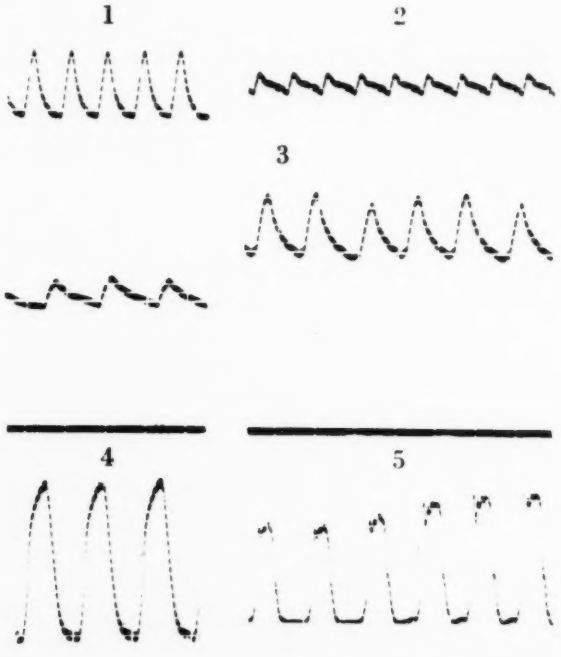

Fig. 1. Records taken with overdamped or slow systems. Systemic and diastolic pressures are given in millimeter Hg. The heart rate is given per minute. *1*—carotid of canary, 250/160, H.R. 570; *2*—external carotid of robin, 110/80, H.R. 620; *3*—carotid of sparrow with zero base line, before excitement 180/140, H.R. 510, after excitement 310/225, H.R. 590; *4*—left ventricle of canary, 150/0, H.R. 735; *5*—left ventricle of robin, before excitement 125/0, H.R. 520, excited 160/0, H.R. 570.

the tip. Manometers equipped with these glass cannulae had frequencies from 220 to 350 cycles per second, which proved adequate to record pulse contours in small animals. However, those equipped with 26 G. needles were either aperiodic from overdamping or had frequencies of the order of 90 c.p.s. and were inadequate to record accurately the pulse contours in animals with heart rates of 500 to 900 per minute.

The overdamped manometers failed to follow quick changes in pressure as shown by the smooth curves of figures 1, *1*, *2*, *3*, *4*. Truer curves, showing all details and taken with a high frequency manometer, can be seen for comparison in figure 2. A record taken with a manometer of too low frequency is seen in figure 1, *5*. The vibrations at the top of the robin's intraventricular pressure curve are of instrumental origin. These records cannot be used to portray pulse contours but are adequate to measure systolic and diastolic pressures with reasonable accuracy.

METHOD. Ventricular pressures were taken by simply inserting the needle through the body wall and into the ventricle. In taking the records from the carp the animal was kept immersed in water. A trephined

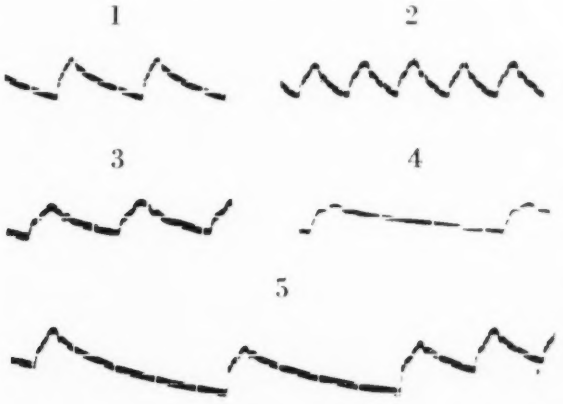

Fig. 2. Carotid records taken with high frequency systems. These show the details of the pressure changes. Values are given as in figure 1. *1*—starling, 210/160, H.R. 375; *2*—mouse, 160/120, H.R. 590; *3*—rat, 180/140, H.R. 430; *4*—pigeon, 140/100, H.R. 200; *5*—mouse while trachea is occluded, 150/100, H.R. irregular.

opening in the plastron allowed access to the turtle's heart without loss of blood or interference with respiration.

Exposure of the brachial, femoral, or carotid arteries (with 1 per cent procaine anesthesia) were made under a binocular loupe. The cannula was inserted and tied in place with the aid of brass wires fastened to flexible leaden supports which stretched, fixed, and occluded the artery. A small glass tube with a loop of thread through it and around the vessel served to stop the flow in some arteries where the brass wires could not be conveniently placed.

General anesthesia (urethane or ether) was used in twelve mice and one rat.

Using low power magnification and a vernier scale, the blood pressure

values could be read from the optical records to within ±1 mm. Hg and the time values to within ±0.5 sigma. The rate of descent of the arterial pressure during diastole was calculated from pressure readings at definite time intervals during a diastole by assuming that a short portion of the curve was linear. The calculated rate of descent was said to occur at the average pressure during that time interval. Where possible readings were made for ten or more time intervals during a single diastole.

TABLE 1

Heart rates and blood pressures of small animals

ANIMAL	NUMBER OF ANIMALS	HEART RATE			BLOOD PRESSURE					
					Systolic			Diastolic		
		Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum
Mouse	*3 V.	620	570	660	112	90	125			
	*9 C.	583	420	720	113	95	125	81	67	90
	2 C.	630	630	630	147	133	160	106	102	110
Rat	*1 C.	425			145			105		
	2 C.	420	410	430	187	180	195′	138	135′	140
Pigeon	4 B.	221	145	290	135	120	140	105	100	115
Starling	2 C.	388	375	400	180	150	210	130	100	160
Robin	2 V, C.	570	520	620	118	110	125	80	80	80
Canary	2 V.	795	750	840	130	110	150			
	4 C.	690	570	840	220	200	250	154	150	160
Sparrow, adult	1 C.	550			180			140		
Sparrow, fledgeling stage	2 V.	502	495	510	123	115	130			
Sparrow, pin feather stage	3 V.	363	330	390	108	80	135			
Frog	6 V, C.	90	75	124	43	36	56	31	24	44
Turtle	11 V, C.	68	60	75	44	38	55	37	28	47
Carp	3 V.	75	72	78	43	40	45			

The cannula was in the carotid artery (C); in the brachial artery (B); and in the ventricle (V). The animals marked (*) were anesthetized. The figures marked (′) were taken 15 minutes after the withdrawal of ether from the anesthetized rat whose readings are shown.

The period of systole was taken to begin with the rise of the arterial pulse curve and to end with the bottom of the incisura. The rest of the cycle including the isometric periods of contraction and relaxation was called diastole. No attempt was made to subdivide the cardiac cycles taken from the ventricle, nor those whose incisurae were not clearly marked.

RESULTS. *Systemic blood pressures.* The systolic and diastolic blood pressures of a number of small animals are given in table 1. With the

exception of those for the pigeon and the cold blooded animals, these values are higher than the mean blood pressures that have been reported in the literature (1, 2, 3). Previous workers used anesthetized animals. Our anesthetized rats and mice showed pressures some 23 per cent lower than our own animals whose pressures were taken when the artery was exposed under local anesthesia. But these anesthetized animals had pressures considerably higher than figures previously reported for the same species. Anesthesia therefore does not account for the whole of the difference. We feel that our animals had less shock than those whose pressures were taken by earlier methods.

Blood pressure is the resultant of cardiac output on the one hand and peripheral resistance on the other. In small animals these quantities must differ from those of larger animals, since metabolism per unit weight

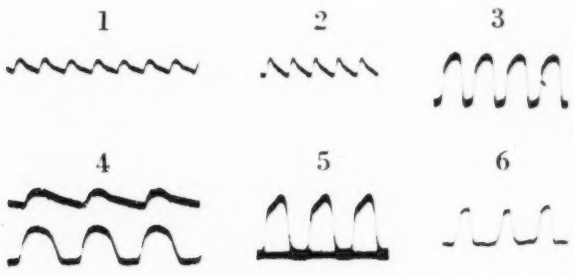

Fig. 3. Records from cold blooded animals. Pressures are given in millimeter Hg. *1*—sciatic artery of frog, 36/24; *2*—truncus arteriosus of frog, 55/40; *3*—ventricle of frog, 45/0; *4*—simultaneous carotid, 40/27, and ventricle, 40/0, of turtle; *5*—ventricle of turtle, 45/0; *6*—ventricle of carp, 40/0.

of body tissue makes a higher demand for blood. Yet the pressures in mice and men are the same. Moreover pressures in dogs under basal conditions (unpublished work) are much higher than those in men. Small birds have higher pressures than those of small mammals. Body size and the problem of transport through the larger arteries are not important in determining the blood pressure level. It may be that animals adapted for sustained activity at a high level (dogs, birds) have higher pressures and the cardiovascular apparatus is thus adapted to hard work. Age may play a rôle as immature animals seem to have lower pressures (see sparrows, table 1).

Relation between systemic and pulmonary blood pressures. Using two manometers, tracings were taken simultaneously from the two ventricles of a mouse anesthetized with urethane. As shown in figure 4, the blood

pressures have approximately the same relationships as are found in larger animals. The contours of pressure are clearly shown and these vary with the intrathoracic pressure changes.

Pulse contours. In small animals the pulse contours are similar to but differ in detail from those of larger animals. The carotid records in figure 2 from small warm blooded animals are more central in type than those of Wiggers (9), taken from the carotids of the dog and man. They resemble more his tracings from the ascending aorta of the larger animals in that the anacrotic halt on the ascending limb of the curve is very plain and that there are very few elastic vibrations of vascular origin. These differences probably result from the relatively large volume elasticity coefficient of these minute arteries.

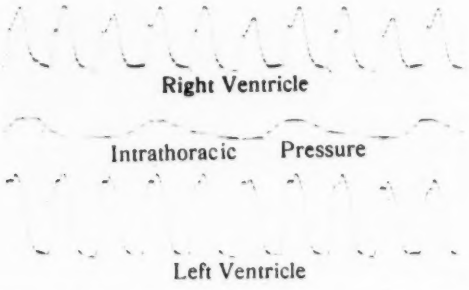

Fig. 4. Simultaneous pressure records from mouse. This shows the effects of respiration upon the contours and pressures within the ventricles. Values given as in figure 1. Right ventricle, 50/0; left ventricle, 125/0; H.R. 660.

Rate of descent of arterial pressure during diastole. Absence of standing waves during diastole brought forcibly to the eye the regularity of the descent of arterial pressure during this period. A glance at figure 2, 5, shows the descent is rapid at first and then gradually becomes less rapid. When the diastole of the first cycle of this record is plotted, pressure against time, the curved line in figure 5 appears. The similarity of this curve in all species justified further examination of the relationship.

Replotting the same cycle, logarithm of the rate of descent of pressure in millimeters Hg per second against pressure in millimeters Hg, the straight line in figure 5 appears. This means

$$\log dp/dt = K P + C, \tag{1}$$

where $\log dp/dt$ is the logarithm of the rate of descent of pressure, K is the slope of the linear relationship, P is the pressure, and C is a constant not yet evaluated.

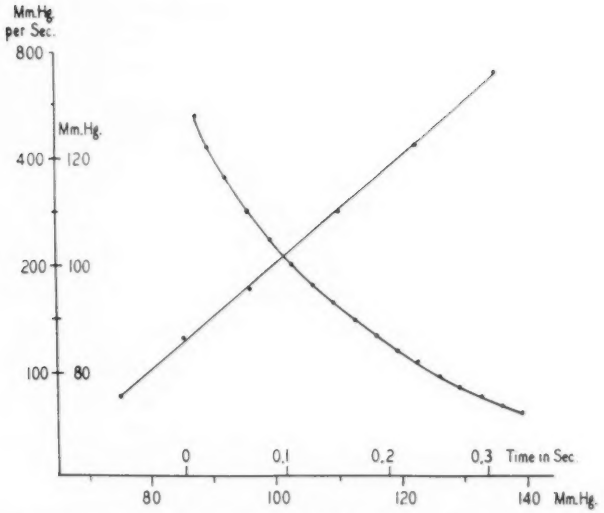

Fig. 5. Descent of arterial pressure during diastole. This is a replot of the first cycle in figure 2, *5*. Curved line: ordinates pressure millimeter Hg, abscissae time in 0.1 second; straight line: ordinates rate of pressure descent during diastole in millimeter Hg per second (logarithm scale), abscissae pressure in millimeter Hg.

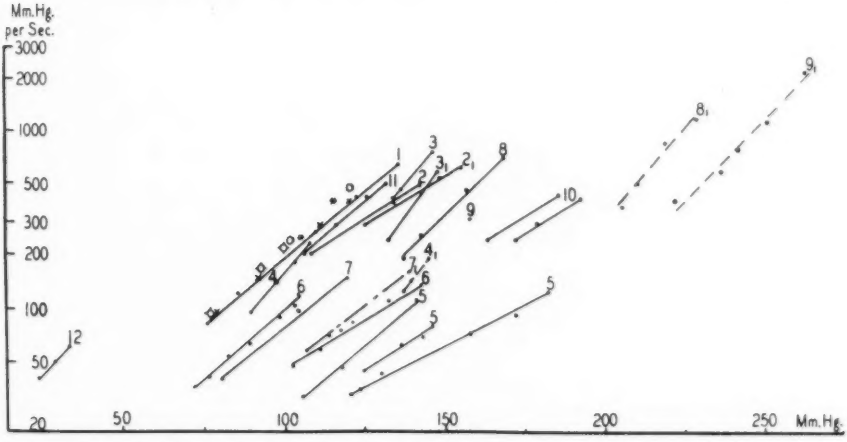

Fig. 6. Relation between rate in millimeter Hg per second of descent of arterial pressure during diastole (ordinate, logarithm scale), and pressure in millimeter Hg (abscissae). Each dot represents a separate determination of both quantities. They are connected by lines representing changes occurring during a single diastole. *1*—mouse, cycles from figure 3, *5*. The points from the first cycle are connected by a line. Separate symbols are used for each of the other cycles: *2–2′* same rat at at different times: *3–3′* two curves from another rat: *4*—rabbit: *4′*—same rabbit excited: *5*—three dogs: *6*—two men: *7*—man, apparently normal: *7′*—same man after 0.2 mgm. epinephrine HCl intravenously: *8*—canary: *8′*—same canary excited: *9*—sparrow, average of several measurements: *9′*—same sparrow excited: *10*—two curves from the same starling: *11*—pigeon: *12*—frog.

Many such relationships have been plotted for various animals, see figure 6. In each case equation 1 is true and the value of K remained contant for all pressures during any one diastole. In different animals it ranged from 0.011 to 0.028.

Otto Frank in 1899 (10) assumed that

$$-dp/dt = \frac{EP}{W} \tag{2}$$

where $-dp/dt$ was the rate of descent of pressure, E was the volume elasticity coefficient, P the pressure, and W the resistance.

From this we obtain the following:

$$\log dp/dt = \log E + \log P - \log W \tag{3}$$

Whittaker and Winton (11) have shown that W may be considered constant and independent of such pressure changes as occur during any single pulse contour. With W constant,

$$\log dp/dt = \log E + \log P \tag{4}$$

Combining equations 1 and 4 we have the following:

$$\log E = K P - \log P + C. \tag{5}$$

The limitations of accuracy in the evaluations and plotting of the relationship between dp/dt and P, limits equation 5 to an approximate evaluation of the relationship between E and P over the pressure range studied.

Further study of figure 6 shows first that the slopes K of the lines marking the linear relationships are similar in different animals; second, that exciting an animal (or injecting epinephrine) increases the blood pressures but does not proportionally increase the rate of descent or even affect K. This indicates that the volume elasticity coefficient of the arterial system varies in the same manner under different physiological conditions and at elevated blood pressures, and in a similar manner in all the animals studied even at the low pressure values of cold blooded animals.

Looking at the chart as a whole we see the dots scattered rather widely but definitely about a diagonal which stretches from the placid frog to to the excited sparrow. The lines indicating relationships within single diastoles fall more rapidly with descending pressures than does this imaginary diagonal.

Throughout diastole the arterial pressure in small animals descends more rapidly than in large animals. This can be seen from the chart and more clearly from column 1, table 2. Two factors can be held responsible: 1, the relatively greater arteriolar outflow, corresponding with the high metabolic rate and the rich capillary blood supply (12) of small animals;

2, the smaller arterial reservoir (Windkessel) in proportion to the size of the animal.

Measurements by Clark (3, b, c) indicate that small animals have a large aortic cross section area in proportion to their weight. None the less, the blood volume within the large named (elastic) arteries is small in proportion to the weight of the animal because these arteries are short.[2] If the length of these arteries is taken as proportional to $\sqrt[3]{\text{body weight}}$ and this is multiplied by the cross sectional area of the aorta in various animals, we have a series of numbers which are more or less proportional

TABLE 2

Comparative relationships

ANIMAL	RATE OF DESCENT OF B.P. LATE IN DIASTOLE	RATIO OF ARTERIAL VOLUME TO BODY WEIGHT*	HEART RATE PER MINUTE	DURATION OF DIASTOLE	DURATION OF SYSTOLE	PER CENT OF CYCLE OCCUPIED BY SYSTOLE	MUSCLE FIBER DIAMETER IN SIGMA	
							a	*b*
	mm. Hg/sec.			*seconds*	*seconds*			
Canary	430		660	0.052	0.039	43		
Sparrow	450		665	0.059	0.037	40		6
Robin			620	0.065	0.032	33		
Starling	250		375	0.117	0.043	27		
Pigeon	200		200	0.235	0.065	21		
Mouse	480	2.1	600	0.057	0.043	43		7
Rat	240	2.6	420	0.085	0.055	40	11.2	
Rabbit	100	2.7	200	0.18	0.12	40	14.5	11
Dog	35	9.6	70	0.56	0.3	35	16.8	14
Man	40	10.0	75	0.53	0.27	34	16.2	

* $\dfrac{\text{Aortic cross section} \times \sqrt[3]{\text{wt.}} \times \text{K}}{\text{Body weight}}$ (see text).

a. Harrison, Ashman and Larson.

b. Our own figures.

to the size of the arterial reservoir (Windkessel) in different animals. As seen from table 2, column 2, these numbers per unit of weight increase with the size of the animal. In small animals a relatively small "Windkessel" is essential, otherwise the heart and blood quantity would be disproportionally large.

Duration of systole and diastole. In order to maintain an adequate mean arterial pressure and particularly to maintain an adequate coronary (diastolic) pressure, the period of diastole in these small animals must be very short. From table 2, columns 1 and 4, it is seen that the duration of

[2] It would be hazardous to extend this reasoning to the small unnamed arteries within organs. These are less expansible than the large arteries.

diastole decreases as the rate of descent of diastolic pressure increases. Harrison, Ashman and Larson (13) have shown that short diastoles are associated with thin cardiac muscle fibers. Indeed the logarithm of the average distance over which oxygen must diffuse ($\frac{1}{4}$ fiber diameter) and the time allowed for diffusion (diastole) are directly proportional. To the series of the Nashville workers we are able to contribute measurements for the mouse and the sparrow which, allowing for differences in technique, fall closely in line.

The percentage of the cycle occupied by systole is greater for the smaller animals than for the larger ones with slow pulse rates. This tendency reaches its limit at a figure of 40 to 45 per cent in the canary and sparrow in case of the birds, but in case of the mammals the limit is reached with the rabbit, mice and rats showing the same figures. What physiological mechanism may set this limit is hard to say. Its usefulness is apparent in that encroachment by systole upon the period of diastole interferes with coronary flow and cardiac filling.

Physiological experiments on small animals. Asphyxia could easily be produced in the mouse by occluding the trachea (see fig. 2, *5*). The heart became slow and irregular and the blood pressure was increased and fluctuating. When the preceding diastole was long and the heart well filled the systolic pulse contours changed decidedly. The initial pressure rise became steeper and the anacrotic halt became higher.

The blood supply to the brain of the robin is unusual in that occlusion of a single median artery in the neck brings on immediate signs of asphyxia. There is no effective collateral circulation.

The arterial pressures in the unanesthetized animals may be above their basal level because exposure of the artery caused excitement and fear. Further excitement, however, increased the blood pressure above the resting unanesthetized level. Blowing in the face of a sparrow increased its blood pressure from 180/140 to 310/225 mm. Hg. Similar changes occurred in the robin (see fig. 1, *5*) and to a lesser degree in the mouse and the rat. Pressure taken by ventricular puncture from unanesthetized canaries gave lower figures than those from the exposed carotid. Ventricular puncture usually produced less fear and excitement.

In addition to increasing the arterial pressure, excitement quickened the heart (28 per cent),[3] shortened both diastole (32 per cent)[3] and systole (17 per cent),[3] and increased the rate of descent of blood pressure during diastole (60 per cent).[3] In one sparrow (not included in above percentages) excitement increased the rate of descent from 600 mm. Hg to 2400 mm. Hg per second. In every case the increase was less than would be predicted by extending the line (fig. 6) to the proper pressure values.

[3] These figures are the average of one observation on each of the following animals before and after attempts to excite them: pigeon, sparrow, canary, and mouse.

Vasoconstriction, which has increased the pressure, decreased the rate of arteriolar outflow and hence the rate of diastolic descent. When the increased blood pressure has resulted from an increase in the stroke volume alone, the line goes upward in its original direction, see figure 6, curve *1*.

SUMMARY

A method is described for taking accurate optical manometric records of arterial and intraventricular blood pressure curves in small animals. These records could be taken without anesthesia and often without injury to the animal.

Pressure pulse contours with systolic and diastolic blood pressure values are presented from the following animals: mouse, rat, canary, sparrow, robin, pigeon, frog, turtle and carp. These are compared with similar curves from larger animals.

The blood pressure level is characteristic of the species and not of the size of the animal. It is higher in small birds than in small mammals; it is the same in mice and man, but higher in dogs and lower in cold blooded animals.

In warm blooded animals the rate at which arterial pressure descends during diastole is inversely correlated with the size of the animal, the relative volume of the "Windkessel" and with the length of diastole. It is directly correlated with the pressure at the time of measurement in all animals. In a single diastole the logarithm of the rate of descent has a linear relationship with pressure.

The volume elasticity coefficient of the arterial system varies with pressure in a constant manner in any individual animal even though marked changes are made in the pressure and in the peripheral vascular system. All animals that have been studied show a similar relationship. An equation is presented describing the approximate relationship between the pressure and the volume elasticity coefficient.

Pressure values, pulse contours, heart rates, length of systole and diastole, rate of pressure descent during diastole, and the effects of pressure changes upon the volume elasticity coefficient were studied when animals were under the influence of excitement, asphyxia and anesthesia.

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EFFECT OF OESTRUS (PSEUDOPREGNANCY) AND CERTAIN PITUITARY HORMONES ON THE LIFE-SPAN OF ADRENALECTOMIZED ANIMALS

W. W. SWINGLE, W. M. PARKINS,[1] A. R. TAYLOR, H. W. HAYS AND J. A. MORRELL

Biological Laboratory, Princeton University, Princeton, N. J. and Research Laboratory, E. R. Squibb & Sons, New Brunswick, N. J.

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A. Oestrus and pseudopregnancy. Rogoff and Stewart (1928) first demonstrated that the bitch, if adrenalectomized when in heat, could survive in normal health and without treatment of any kind for as long as sixty-five days. This observation has been repeatedly confirmed in this laboratory by withholding cortical hormone from the adrenalectomized bitch in heat (Pfiffner, Swingle and Vars, 1934; Swingle, Parkins, Taylor and Morrell, 1936).

The effect of oestrus (and pseudopregnancy) upon the life-span of the adrenalectomized bitch not receiving cortical hormone is so striking that we undertook an investigation of the problem. The work was greatly facilitated by discovery of a simple and effective method for experimentally inducing oestrus and pseudopregnancy in this species.

We have studied the effect of oestrus, both spontaneously occurring and experimentally induced, upon the life-span of eight adrenalectomized dogs not receiving cortical hormone. The animals had been operated for long periods and most of them had been studied through several cycles of adrenal insufficiency and recovery on extract. The effect of oestrus and pseudopregnancy upon the life-span of these animals is shown in table 1.

Various estrogenic and gonadotropic hormones were tested on dogs in an effort to devise a rapid and uniformly successful method of inducing oestrus in this species. It has been known for some years that extracts of castrate and menopause urine are extremely rich in follicle-stimulating hormone of the anterior pituitary and appear to contain but little of the luteinizing factor. Several workers have demonstrated that this material is effective in inducing oestrus in the rat. Material prepared by one of us (J. A. M.) has invariably produced complete oestrus in mature, adrenalectomized bitches within a week to ten days when given in daily doses equivalent to 50 R. U. We were able to induce oestrus in two immature animals by

[1] E. R. Squibb and Sons Fellow in the Biological Sciences.

giving large doses. The criteria adopted for determining oestrus was the presence of numerous cornified cells in the vaginal smear (Evans and Cole, 1931), and copulation with a vasectomized male. Following mating the injections of menopause urine extract and cortical hormone were discontinued. We have not observed any difference between oestrus and pseudopregnancy occurring spontaneously and that induced experimentally. In so far as the effect upon the life-span of the adrenalectomized animals is concerned both types are identical.

The animals when taken off cortical hormone remain healthy and vigorous and usually develop voracious appetites. Table 1 shows the weight increases which occur during the pseudopregnant period. The onset of adrenal insufficiency symptoms in these dogs is seldom abrupt. As the period draws to a close, the serum sodium and chloride and arterial pres-

TABLE 1

Effect of oestrus and pseudopregnancy upon the life-span of the adrenalectomized bitch not receiving cortical hormone

DOG NUMBER	TYPE OESTRUS, SPONTANEOUS OR INDUCED	WEIGHT	DAYS OFF EXTRACT	WEIGHT	MENOPAUSE URINE EXTRACT DAILY DOSE*
		kgm.		*kgm.*	
1	Induced	8.6	43	10.4	50 r.u. for 7 days
2	Induced	9.7	58	12.9	50 r.u. for 6 days
3	Induced	7.2	52	8.5	50 r.u. for 7 days
4	Induced	9.3	50	9.0	50 r.u. for 8 days
5	Induced	10.1	40	10.7	50 r.u. for 7 days
6	Spontaneous	9.5	60	10.8	
7	Spontaneous	10.1	45	10.0	
8	Spontaneous	8.6	52	8.8	

* Menopause urine extract given subcutaneously along with the usual maintenance dose cortical hormone until onset of oestrus.

sure gradually decline. The hemoconcentration increases and symptoms appear. During the latter part of pseudopregnancy the blood picture of the animals not receiving extract resembles that of a dog kept on a subminimum maintenance dose of cortical hormone. The animals are free from obvious symptoms but activity and vigor gradually decline and the appetite fails. Cortical hormone was injected intravenously when marked symptoms appeared. Two animals lactated copiously for a week to ten days at the close of pseudopregnancy and after receiving injections of cortical hormone.

Reasonably complete blood studies were made on four animals at intervals throughout the cycle, and the data obtained from two cases representative of experimentally induced oestrus and pseudopregnancy are given in table 2. In general, the blood picture remains normal during the period of

good health. The blood urea nitrogen and blood glucose tend to remain elevated through the cycle. The interesting thing, however, is the maintenance of normal blood dilution and serum electrolyte levels for such long intervals in the absence of any treatment such as salt feeding or cortical hormone administration.

TABLE 2

Blood changes in the adrenalectomized bitch (not receiving cortical hormone) during oestrus and pseudopregnancy

DATE	WEIGHT	PULSE	BLOOD PRESSURE	SERUM SODIUM PER LITER	SERUM CHLORIDE PER LITER	HEMOGLOBIN	RED BLOOD CELL VOLUME	R.B.C.	UREA	SUGAR	REMARKS
A. Dog 2—58 days without cortical hormone											
	kgm.	*min.*	*mm. Hg*	*m.-eq.*	*m.-eq.*	*grams per cent*	*per cent*	*mil.*	*mgm. per cent*	*mgm. per cent*	
2/11/36	9.7	68	108	141.0	114.4	10.5	35.4	4.43	25	84.5	Oestrus. Hormone discontinued
3/ 2/36	10.8	98	100	140.9	114.0	10.8	36.8	5.76	44.4	80	Normal health
3/12/36	12.5	96	95	142.6	114.0	11.9	34.6	5.21	32.7	93.5	Normal health
3/23/36	12.5	112	92	140.6	113.8	13.3	41.0	6.40	38.4	89	Normal health
4/ 2/36	12.8	108	94	141.2	114.2	13.5	44.0	6.60	40.8	91.5	Normal health
4/ 9/36	12.9	52	75	133.5	112.8	12.5	41.6	6.57	86.9	89	Mild symptoms, hormone injected
B. Dog 3—52 days without cortical hormone											
3/ 4/36	7.2	108	93	138.7	109.2	11.7	38.3	5.54	27.7	100	Oestrus. Hormone discontinued
3/18/36	8.0	104	87	141.4	110.9	12.4	35.5	5.53		105	Normal health
3/28/36	8.6	124	88	137.1	108.2	13.5	39.7	7.20	27.7	105.6	Normal health
4/ 8/36	8.6	140	82	131.2	105.8	14.0	44.8	7.41	33.9	102.5	Poor appetite
4/18/36	8.9	172	74	118.2	101.0	13.9	44.0	8.48	41.6	111.5	Lethargic
4/25/36	8.5	92	48	111.0	86.7	14.0	45.5	6.55	71.4	87	Severe symptoms, hormone injected

Dog 3, table 2 B, is of considerable interest in that the serum sodium and chloride (late in the period) fell to very low levels (below those characteristic of uncomplicated adrenal insufficiency) and remained low for eight days without marked symptoms appearing. The writers have shown that it is possible to maintain adrenalectomized dogs at low serum electrolyte levels

on a salt-free diet by administering large daily doses of cortical hormone (Swingle, Parkins, Taylor and Hays, 1936). However, this dog received no treatment whatever.

Attempts were made to prolong the life-span by using various hormones and hormone combinations but, as shown in table 3, with the possible exceptions of dogs 8 and 9, the results are essentially negative. It is of

TABLE 3

Effect of various hormones and hormone combinations upon the life-span of the adrenalectomized dog not receiving cortical extract

EXPERIMENT	TREATMENT GIVEN	DURATION EXPERIMENT	REMARKS
		days	
1A	100 u. follutein* plus 100 u. antex† daily	5	Severe symptoms. Cortical hormone injected
2A	1 int. u. proluton‡ plus 50 u. follutein daily	8	Severe symptoms. Cortical hormone injected
3A	2 int. u. proluton plus 50 u. follutein daily	4	Severe symptoms. Cortical hormone injected
4A	2 int. u. proluton daily	12	Severe symptoms. Cortical hormone injected
5A	100 u. follutein plus 40 u. prolactin daily	5	Severe symptoms. Cortical hormone injected
6A	2 cc. Antuitrin S§ daily	6	Severe symptoms. Cortical hormone injected
7A	5 cc. anterior pituitary extract (Squibb) daily or 0.5 cc. per kgm.	7	Severe symptoms. Cortical hormone injected
8A	50 u. follutein plus 40 u. extract menopause urine daily	18	Severe symptoms. Cortical hormone injected
9A	20 cc. urine pregnancy (untreated) intraperitoneally	19	Ovariectomized animal. Died suddenly

* Follutein is a commercial preparation of anterior pituitary-like substance from pregnancy urine (Squibb).

† Antex is a follicle stimulating substance from pregnant mares' serum obtained through the courtesy of Dr. C. Hamburger of Copenhagen.

‡ Proluton is a commercial preparation of crystalline progesterone in oil (Schering).

§ Antuitrin S is a commercial preparation of anterior pituitary-like substance from pregnancy urine (Parke Davis).

course possible that larger doses of the materials employed might have produced greater effects. Dog 9, an ovariectomized animal, receiving unextracted urine of pregnancy, remained in fair condition for 19 days and was found dead on the morning of the 20th day after discontinuting cortical hormone. The urine was Seitz filtered before injecting it intraperitoneally.

B. Effect of anterior pituitary extract upon the life-span of bilaterally adrenalectomized cats. The striking effect of oestrus and pseudopregnancy on

the life-span of the adrenalectomized bitch indicated the possibility that the anterior pituitary might be an important factor in maintaining normal health in these animals. Wilder (1934) stated, in a brief report, that A. P. extract could be employed with advantage as a supplement to adrenal cortical hormone in the treatment of Addison's disease. The material used by him was a preparation manufactured by E. R. Squibb & Sons. We therefore investigated the effect of this commercial product upon the life-span of adrenalectomized cats not receiving cortical extract (Swingle, Parkins, Taylor and Morrell, 1936).

The hormone used is an alkaline aqueous extract of cattle anterior pituitary. Besides the growth factor, it contains appreciable amounts of thyrotropic, adrenotropic and gonadotropic factors. It is prepared by a modification of the method originally described by Van Dyke and Wallen-Lawrence (1930). One unit of this growth-promoting component of anterior pituitary extract (Squibb) is the amount which when injected each day for ten consecutive days into female rats (weighing 250 ± 25 grams) will cause an increase of 10 per cent in body weight within eleven days.

The cats were bilaterally adrenalectomized and maintained in normal health and vigor for ten days by daily injections of cortical hormone. The extract was then withdrawn and the animals permitted to develop severe symptoms of insufficiency. Cortical hormone was then injected, the animals revived and restored to normal, and 1 cc. per kgm. of anterior pituitary extract given daily subcutaneously, along with the usual maintenance dose of cortical hormone. At the end of three days the cortical hormone was withdrawn so that the animal received only the A. P. extract until the end of the experiment. The effect of this treatment in lengthening the life-span of the cats is shown in table 4.

Examination of the data shows that the survival period of the adrenalectomized cats receiving growth hormone is considerably longer than the usual life-span of this species when the adrenals are removed and cortical extract withheld. Bilateral adrenalectomy in the cat usually terminates fatally within the first seven to eleven days following removal of the second gland where the two stage operation is used, and several days earlier if both adrenals are extirpated at a single stage operation. Some of the figures cited in the literature for the average survival of untreated cats following a two stage adrenalectomy are as follows: 11 days (Rogoff and Stewart, 1927); 7.7 days (Swingle and Pfiffner, 1932) using 38 animals, and 8.6 days in a series comprising 100 animals; 7.5 days (Britton, 1930). Macroscopic accessory glands are uncommon in this species.

Blood studies were not made since the animals are sensitive to small blood loss. In general the cats remained active and vigorous for the first two weeks after withdrawal of cortical hormone. After this interval, they

usually (though not invariably) exhibited signs resembling those of chronic insufficiency produced by a sub-minimum maintenance dose of cortical hormone. The appetite and vigor gradually declined, slight weakness of the hind legs developed, the hair coat became rough and dry and tended to shed easily. This condition persisted for several weeks in some of the cats and was associated with a sharp decline in weight. This slowly developing cachexia did not always appear, for several animals maintained for long periods on A. P. extract remained fairly active and vigorous and with appetite unimpaired until a few days before death. Three cats showing cachexia after four weeks' maintenance on A. P. extract, developed hypoglycemia. They were revived from convulsions by a single injection of

TABLE 4

Effect of anterior pituitary extract upon the life-span of bilaterally adrenalectomized cats not receiving cortical hormone

CAT NUMBER	SEX	DAILY DOSAGE PER KGM.	SURVIVAL PERIOD	REMARKS
		cc.	*days*	
1	♂	1 3	21	Marked cachexia
2	♀	1 0	35	Cachexia. Terminal hypoglycemia
3	♂	1 0	150	Anterior pituitary extract discontinued. Severe insufficiency in 4 days
4	♀	1 0	21	Cachexia
5	♀	1 0	28	Cachexia
6	Castrate ♂	1 0	25	Feline distemper. Pneumonia
7	♀	1 0	21	Cachexia
8	♀	1 0	30	Typical adrenal insufficiency
9	♂	1 0	40	Symptoms. Hypoglycemia at death
10	Castrate ♂	1 0	47	Severe adrenal symptoms. Cachexia. Hypoglycemia
11	♀	1 0	13	Adrenal insufficiency
12	♂	1 0	14	Feline distemper. Pneumonia

glucose and survived a few days longer. The following two protocols give the essential data on a male and female. Cat 3 is exceptional in that he was the only animal in which growth hormone was discontinued until symptoms developed and then resumed again; he is also exceptional in regard to the unusually long survival period.

Protocol 1. Cat 3. Adult male, weight 2.5 kgm. Bilaterally adrenalectomized 9/28/35. Given cortical hormone daily until 11/8/35. Cortical extract discontinued and 1 cc. per kgm. body weight of anterior pituitary extract given daily. 11/27/35 animal in excellent health. Weight 2.8 kgm. Had not received cortical hormone for 50 days. Anterior pituitary extract discontinued on this date. 12/1/35, symptoms of adrenal insufficiency. Animal weak and spastic. Weight 2.7 kgm. Anterior pituitary extract injections resumed. 1/25/36, animal in excellent condition, is

active and playful. Weight 3.1 kgm. Anterior pituitary extract injections stopped. 1/28/36, definite symptoms of adrenal insufficiency apparent. Weight 3 kgm. Anterior pituitary extract injections resumed. 2/25/36, animal active and vigorous. Weight 3.2 kgm. 3/3/36, anterior pituitary extract withdrawn. 3/6/36, animal prostrate from adrenal insufficiency. Death weight 2.9 kgm. Autopsy showed no accessory glands. Total survival 150 days.

Protocol 2. Adult female. Weight 2.5 kgm. Bilaterally adrenalectomized and permitted to develop marked symptoms of adrenal insufficiency. Revived and restored to normal health with cortical hormone. 11/27/36 to 11/29/36, given anterior pituitary extract 1 cc. per kgm. daily along with maintenance dose of cortical hormone. 11/30/36, cortical extract discontinued. Weight 2.5 kgm. Normal health and vigor. 12/10/36, cat active and playful. Weight 2.4 kgm. 12/23/36, no symptoms. Weight 2.2 kgm. 12/29/36, animal weak and refuses food. 12/30/36, death. Autopsy showed no accessory adrenals. Total survival on anterior pituitary extract 30 days.

Effect of the anterior pituitary extract upon the life-span of the castrate adrenalectomized male cat. In view of the effect of A. P. extract upon the survival period of adrenalectomized male cats (table 4) it seemed evident that the female sex glands and their hormones could not be the agents responsible for lengthening the life-span, but the question of the possible influence of the male sex hormones remained. In order to determine the influence of the testes, two adult adrenalectomized males were castrated. These animals had previously been allowed to develop adrenal insufficiency and were restored to normal with cortical hormone. Upon recovery from the operation cortical hormone was withdrawn and 1 cc. per kgm. of A. P extract given daily. One animal survived in excellent health for twenty days. On the twenty-third day he developed symptoms of feline distemper—watery eyes, plugged nose and labored breathing. He died on the 25th day after discontinuing cortical hormone. The protocol of the other castrate is given below.

Protocol 3. Adult male. Bilaterally adrenalectomized and permitted to develop adrenal insufficiency. Restored to normal with cortical hormone and castrated under ether anesthesia after first priming the animal with cortical hormone. Upon complete recovery from the operation, cortical extract was withheld. Beginning on 12/4/36, 1 cc. per kgm. of A.P. extract given daily. Animal in excellent health. Weight 3.3 kgm. 1/4/37, animal fairly active, eats well and is playful but shows weakness in hind legs when walking. Weight 3.3 kgm. 1/11/37, condition unchanged. Weight 2.9 kgm. 1/17/37, animal weak and spastic. Hair coat is rough and the hair falls out easily. Weight 2.8 kgm. Appetite failing. Died 1/19/37, on 47th day after discontinuing cortical hormone. No cortical tissue could be found at autopsy.

It is obvious from these castration experiments that the anterior pituitary extract used does not act through the mediation of the male sex glands in prolonging the life-span of these cats.

Effect of the corpus luteum hormone, progesterone, upon the life-span of the

intact and castrate adrenalectomized cat. Rogoff and Stewart (1928) in their original experiments on the effect of oestrus on the adrenalectomized dog, called attention to the possibility that the corpus luteum might be involved. This seemed a logical conclusion considering that pseudopregnancy in the dog is associated with the luteal phase of the ovarian cycle. Recently, Emery and Schwabe (1936) were able to show that pituitary implants in adrenalectomized rats ameliorate the symptoms of insufficiency for a period of at least three weeks. They postulated that the corpus luteum was the active agent involved and not the pituitary *per se*.

An investigation of the effect of crystalline progesterone upon the life-span of adrenalectomized cats was undertaken. The material was obtained through the courtesy of Dr. Irwin Schwenk of the Schering Corporation to whom we express our gratitude. Proluton (Schering) is the trade name of a solution of crystalline progesterone in oil. Each International Unit is equal to one milligram of crystalline hormone which is approximately the equivalent of one Corner-Allen unit.

In earlier experiments where non-castrate adrenalectomized dogs were used, the results of injecting Proluton were negative (table 3). Further work was undertaken on castrate male and female cats. Two adrenalectomized cats, a male and a female, were castrated and upon complete recovery from the operation a week later were each given daily 2 International Units of progesterone in oil (Proluton) subcutaneously. One animal survived 6 and the other 7 days following withdrawal of cortical hormone. No toxic or untoward effects of the injections were observed. The experimental evidence does not support the idea that progesterone will lengthen the survival period of castrate adrenalectomized animals. Our previous work had shown the non-effect of corpus luteum hormone on the non-castrate adrenalectomized dog.

Discussion. Oestrus in the dog is followed by a prolonged period of pseudopregnancy which closely approximates the length of the gestation time in this species. The adrenalectomized dog is maintained without cortical hormone during this period. According to present-day knowledge of the subject, pseudopregnancy corresponds to the luteal phase of the ovarian cycle. It is surprising, therefore, to find that the crystalline hormone of the corpus luteum has no influence on the life-span of the adrenalectomized dog or cat. However, it is apparent that the A. P. extract (Squibb) does contain a factor which prolongs the survival period of the adrenalectomized cat. Previous to the use of castrate males as test animals we were inclined to believe that the gonadotropic factors known to be present in this extract were the agents responsible for maintaining the animals (Swingle, Parkins, Taylor and Morrell, 1936). However, since A. P. extract is effective in increasing the life-span of the adrenalectomized castrate it is clear that the action in these cats was not through the mediation of the gonads.

Evans et al. (1933) reported that injections of a highly purified pituitary growth hormone did not increase the survival period of adrenalectomized rats; hence, it would seem that the growth hormone, *per se*, is not the active factor. The evidence from the bitch in heat points to the pituitary gonadotropic factors acting in conjunction with the corpus luteum as the responsible agents. On the other hand, the experiments with crystalline progesterone and the response of the castrate cat to A. P. extract (Squibb) leads one to seriously question the rôle of the sex glands. In these experiments the anterior pituitary is clearly involved, substituting functionally over long periods for the adrenal cortical hormone in the animal lacking adrenals.

SUMMARY AND CONCLUSIONS

1. The adrenalectomized bitch does not require cortical hormone during the period of pseudopregnancy following oestrus. The animal remains active and vigorous during this interval.

2. The serum sodium and chloride levels remain normal during the period of good health. Hemoconcentration and fall in arterial pressure occur when the animal is nearing the end of the pseudopregnant period.

3. Extract of menopause urine is an effective agent in inducing oestrus in the dog.

4. Attempts to prolong the life-span of adrenalectomized bitches (not in heat) by injections of large amounts of various ovarian and pituitary hormones were not successful.

5. Anterior pituitary extract prolongs the life-span of bilaterally adrenalectomized cats. The sex of the animal or the presence or absence of sex glands has no apparent effect on the action of the extract.

6. Daily injections of large amounts of the crystalline hormone of the corpus luteum do not prolong the life-span of bilaterally adrenalectomized cats.

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THE INFLUENCE OF ADRENAL CORTICAL HORMONE UPON ELECTROLYTE AND FLUID DISTRIBUTION IN ADRENALECTOMIZED DOGS MAINTAINED ON A SODIUM AND CHLORIDE FREE DIET

W. W. SWINGLE, W. M. PARKINS,[1] A. R. TAYLOR AND H. W. HAYS

From the Biological Laboratory, Princeton University, Princeton, N. J.

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In previous communications (Swingle, Pfiffner, Vars and Parkins, 1934a, b, c, d; Parkins, Taylor and Swingle, 1935; Swingle, Parkins, Taylor and Hays, 1936a, b) the writers and co-workers have presented evidence that the hemoconcentration and circulatory failure characteristic of adrenal insufficiency are due to an impairment of the mechanism regulating and controlling the *internal* fluid distribution of the body. The experimental data were such as to lead us to reject the view originally advanced by Loeb et al. (1933, 1935) and Harrop et al. (1933) that adrenal insufficiency can be adequately explained by undue loss of body fluid and electrolytes by way of the urine. Recently Harrop and co-workers (1936) have reported that wastage of body water as urine is not a necessary accompaniment of adrenal insufficiency in the dog.

Nevertheless, the fact remained that renal excretion of considerable quantities of sodium and chloride occurs in adrenal insufficiency and is accompanied by a fall in the concentration of these electrolytes in the serum. There can be no reasonable doubt that in the dog, the usual electrolyte changes in uncomplicated insufficiency under ordinary conditions of food intake, are much as Loeb et al. and Harrop et al. have described. There is an increased urinary output of sodium and chloride and retention of potassium; the serum sodium and chloride decline whereas the serum potassium rises. Following hormone injections these conditions are reversed; the serum sodium and chloride rise and the potassium falls; this is accompanied by renal retention of sodium and chloride and elimination of potassium, etc. However, previous experiments in which adrenalectomized dogs maintained at low serum electrolyte levels were run through the complete cycle of insufficiency and recovery on extract treatment without increased renal elimination of sodium and chloride, led us to question the significance of external loss of electrolytes in adrenal

[1] E. R. Squibb and Sons Fellow in the Biological Sciences.

insufficiency. Britton and Silvette (1936, 1937) have reported that the serum concentrations of sodium and chloride are actually increased in marmots and opossums dying of uncomplicated adrenal insufficiency. The following experiments indicate that losses of water and electrolytes to the exterior of the body by way of the urine are not *necessary* accompaniments of adrenal insufficiency in the dog.

METHODS. The dogs used in these experiments were strong, healthy, vigorous animals that had been bilaterally adrenalectomized for periods varying from six months to one year previous to use and maintained in normal condition by adequate daily injections of cortical hormone. The methods used for blood analysis are as follows: Serum sodium, Butler and Tuthill (1931); serum chloride, Van Slyke and Sendroy, as modified by Eisenman (1929); serum potassium, Taylor (1930); blood glucose, Somogyi's modification of the Shaffer-Hartman reagent; blood urea nitrogen, Folin and Svedberg (1930); hemoglobin, Newcomer; cell volume, sealed capillary tubes centrifuged at 20,000 times gravity. The urine sodium method was the same as that used for serum; urine potassium, Taylor's method with modifications as given by Robinson and Putnam (1936) (the samples were ashed); urine chloride, Volhard-Harvey (Peters and Van Slyke, 1932); arterial pressure, Parkins (1934).

The animals were kept in metabolism cages which were washed thoroughly each morning with distilled water. The urine was collected under toluene, and all blood samples were drawn, delivered and centrifuged under oil. The sodium and chloride free diet employed was prepared according to the method described in our previous paper (Swingle, Parkins, Taylor and Hays, 1936b).

A. *Blood and urine studies on the normal intact dog maintained on a sodium and chloride free diet.* Two adult male dogs were studied, but since the experimental results were comparable in the two cases only one will be discussed in detail. The pertinent data are given in table 1. The animal's weight declined 0.6 kgm. during the experiment. The arterial pressure, blood urea nitrogen and blood glucose remained unchanged. The serum sodium and chloride decreased slightly but the change is probably not significant. However, the blood showed evidence of concentration as indicated by increases in hemoglobin, red cell volume and erythrocyte count. The dog remained healthy and vigorous throughout the experiment.

The water balance of this animal showed no unusual changes. Sodium and chloride were freely excreted in the urine the first day the salt-free diet was given but thereafter the daily renal elimination of these ions decreased to mere traces. The daily urine volume and water intake were less during the period the dog was given the sodium and chloride free diet, compared with the fore-period when ordinary meat scraps were fed.

TABLE 1

Electrolyte and fluid distribution of a normal dog maintained on a sodium and chloride free diet

DATE	PULSE PER MINUTE	BLOOD PRESSURE	SERUM SODIUM	SERUM CHLORIDE	HEMOGLOBIN	RED CELL VOLUME	R.B.C.	UREA N	BLOOD GLUCOSE	WEIGHT	FOOD OFFERED	FOOD INGESTED	WATER INTAKE	URINE VOLUME*	URINE SODIUM	URINE CHLORIDE	REMARKS
		mm. Hg	*m.-eq. per liter*	*m.-eq. per liter*	*grams per cent*	*per cent*	*millions*	*mgm. per cent*	*mgm. per cent*	*kgm.*	*grams*	*grams*	*cc.*	*cc.*	*m.-eq.*	*m.-eq.*	
5/26/36	104	106	144.3	111.8	14.8	45.6	6.64	22.7	91	8.8	200	0	240	220	20.4	35.0	Sodium-chloride free diet
5/27/36											200	45	120	0			Sodium-chloride free diet
5/28/36											200	30	290	165	6.8	6.0	Sodium-chloride free diet
5/29/36											150	150	75	0			Sodium-chloride free diet
5/30/36											200	200	190	175	1.4	6.1	Sodium-chloride free diet
5/31/36											200	200	120	0			Sodium-chloride free diet
6/ 1/36	98	104	144.1	111.8	15.1	49.0	7.0	22.2	89	8.3	200	200	130	150	0.3	1.5	Normal health
6/ 2/36											250	250	140	185	Trace	1.1	Sodium-chloride free diet
6/ 3/36											250	250	105	0			Sodium-chloride free diet
6/ 4/36	100	102	142.4	111.2	15.5	50.0	7.10	24.6	87	8.3	250	250	150	180	Trace	0.3	Sodium-chloride free diet
6/ 5/36											250	205	150	245	0.1	1.6	Sodium-chloride free diet
6/ 6/36											225	225	165	150	Trace	0.9	Sodium-chloride free diet
6/ 7/36											250	190	90	175	0.1	1.0	Normal health
6/ 8/36	94	105	142.4	109.8	16.8	52.0	8.30	20.6	87	8.2							Salted food given

* 24 hour urine samples were collected; the first is urine of 5/26/10:00 a.m. to 5/27/10:00 a.m.

B. *Blood and urine studies on the healthy, vigorous, adrenalectomized dog maintained on a sodium and chloride free diet and receiving cortical hormone.* Two adult males were studied and the data on blood, urine and water balance, together with the weight changes and daily dosage of cortical hormone, are given in table 2. These data appear to be of considerable interest and significance.

The serum sodium, chloride and potassium remained practically unchanged from 1/5/37 to 1/20/37, a period of 15 days, during which the dog received only the sodium and chloride free food and cortical hormone. The chloride fluctuated slightly but probably not significantly. During the 15 day interval, the urinary excretion of sodium, chloride and potassium diminished until 1/19/37, when a sudden increased elimination of sodium and chloride occurred (table 2). Despite the fact, the level of the serum electrolytes remained normal following reduction in extract dosage on 1/11/37 and 1/16/37; the blood showed unmistakable signs of concentration as revealed by increase in hemoglobin, cell volume and erythrocyte count. This hemoconcentration was evidently due to reduction of extract dosage. The other adrenalectomized dog of this series of experiments received a daily dose of hormone equivalent to 3 cc. per kilogram of body weight. This dose was not reduced and as a result no sign of hemoconcentration appeared until hormone was discontinued. However, when the injections were stopped the animal became anuric and renal excretion of sodium, chloride and water ceased entirely during the insufficiency period and was resumed during the recovery interval following extract injection.

The blood sugar level of the animal discussed in table 2 flucutated somewhat but remained within the normal range until marked symptoms developed. There was a sharp fall at this time, but not to a hypoglycemic level. The other experimental dog showed a 5 mgm. per cent decrease when in severe insufficiency and an additional 4 mgm. reduction during recovery on massive doses of extract. The blood urea nitrogen varied widely, and in this respect behaved much like it does in the adrenalectomized dog kept on a sub-minimum maintenance dose of extract (Pfiffner, Swingle and Vars, 1934).

On the 15th day of the experiment (1/20/37) the animal appeared listless. His appetite had failed two days previously. Extract injections were then discontinued. At 10:30 p.m. on 1/21/37, the dog was verging on collapse and exhibited all of the well known symptoms of adrenal insufficiency. The arterial pressure was 52 mm. Hg; the animal was weak and spastic and walked with a distinct sway. Blood samples were taken and 4 cc. of cortical hormone per kilo of body weight injected intravenously, plus 1 cc. per kilo intraperitoneally, making a total of 5 cc. per kgm.

TABLE 2

Electrolyte and fluid distribution of an adrenalectomized dog maintained on a sodium and chloride free diet, and studied through a cycle of insufficiency following hormone withdrawal and injection

DATE	PULSE PER MINUTE	BLOOD PRESSURE	SERUM SODIUM	SERUM CHLORIDE	SERUM POTASSIUM	HEMOGLOBIN	RED CELL VOLUME	R.B.C.	UREA N	BLOOD GLUCOSE	WEIGHT	FOOD OFFERED	FOOD INGESTED	WATER INTAKE	URINE* VOLUME	URINE SODIUM	URINE CHLORIDE	URINE POTASSIUM	CORTICAL EXTRACT	REMARKS
		mm. Hg	*m.-eq. per liter*	*m.-eq. per liter*	*m.-eq. per liter*	*grams per cent*	*per cent*	*millions*	*mgm. per cent*	*mgm. per cent*	*kgm.*	*grams*	*grams*	*cc.*	*cc.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*cc. per kgm.*	
1/ 5/37	88	100	139 3	114 4	6 8	13 4	42.6	6.70	32 2	82 0	10 1	350	350	280	294	10 0	14 1	12 6	1 0	Salt-free diet
1/ 6/37												400	400	445	450	2 8	5 4	11 3	1 0	Normal
1/ 7/37	112	92				12 1	39 3	6 14	100 0	87 0	10 1	400	360	475	540	6 5	6 5	13 7	2 0	Normal
1/ 8/37												400	280	440	645	2 3	7 7	18 1	2 0	Normal
1/ 9/37	96	95		113 8		12 8	41 3	5 45	90 6	91 5	10 0	300	135	280	275	1 0	3 9	4 0	2 0	Normal
1/10/37												250	60	235	145	Trace	3 1	5 0	2 0	Normal
1/11/37	76	98		113 2		12 8	39 3	6 28	33 9	94 3	9 8	200	200	265	250	Trace	5 3	5 1	1 5	Normal
1/12/37												250	250	365	455	3 5	6 1	11 6	1 5	Normal
1/13/37	88	92		112 8		14 2	44 8	6 60			9 8	300	300	345	158	0 3	1 6	2 0	1 5	Normal
1/14/37	96	91	139 9	109 6	6 1	13 9	42 3	6 51	86 9	96 0	9 8	350	350	360	333	1 2	2 8	4 9	1 5	Normal
1/15/37												350	95	365	525	1 3	3 8	8 6	1 5	Normal
1/16/37												250	0	300	190	1 3	3 2	3 5	1 2	Normal
1/17/37	116	92				14 2	43 6				9 6	200	80	185	153	1 5	3 0	5 6	1 2	Poor appetite
1/18/37												150	0	180	105	1 1	2 0	4 8	1 2	Refuses food
1/19/37	120	88		111 8		16 3	48 7	7 57			9 4	150	0	275	229	5 0	7 8	6 2	1 2	Somewhat lethargic
1/20/37	120	90	139 7	112 0	5 7	16 7	49 7	8 12	52 6	78 0	9 3	100	0	180	0	0	0	0	0	Extract discontinued
1/21/37												100	0	75	0	0	0	0	0	Extract discontinued
10:30 p.m.	160	52	136 4	110 2	8 3	17 5	50 2	8 07	95 2	66 5	9 2	0	0	160	172	2 4	3 7	10 7	5 0	Marked insufficiency. Extract†
1/22/37	140	83	134 4	108 4	5 6	14 9	43 6	6 44			8 9	100	0	285	360	8 6	14 8	18 6	3 0	No symptoms
1/23/37	128	94	137 4	110 2	5 4	14 0	42 6	6 40	40 8	73 0	8 7	100	0	180	88	1 8	5 3	4 4	3 0	No symptoms Experiment discontinued
1/24/37	112	93										400	400	1,020	335	46 4	51 3	12 8	1 5	Salted food given‡
1/25/37												500	500	955	520	108 2	114 3	12 3	1 5	Salted food given‡
1/26/37	88	92	144 6	119 0	7 1	9 5	29 1	4 36	24 6	87	9 9	500	500	350	390				0 5	Unsalted Wilsco

* 24 hour urine samples were collected. The first is urine of 1/5/10:00 a.m. to 1/6/10:00 a.m.

† 4 cc. per kgm. body weight intravenously and 1 cc. per kgm. intraperitoneally.

‡ 6 grams sodium chloride added to canned dog food (Wilson & Co., Wilsco) on 1/24/36 and on 1/25/36.

Eleven hours later the dog was active, vigorous and eager for food. The blood showed marked dilution.

Examination of the data presented in table 2 shows clearly that changes in the level of the serum sodium and chloride, under the conditions of this experiment, are quite inadequate in themselves to account for the symptoms of adrenal insufficiency following hormone reduction and withdrawal. The fall in serum sodium concentration amounted to but 2.9 m.-eq/l. and in serum chloride 4.2 m.-eq./l; the increase in serum potassium was 1.5 m.-eq./l as compared to the normal for this dog. Moreover, following the initial injection of massive doses of hormone, and in the absence of any food intake, the levels of serum sodium and chloride declined. At this time blood dilution and rise in arterial pressure were marked, and all symptoms had disappeared. The fall in serum sodium and chloride following extract injection is probably more apparent than real. The data in table 2 show that 12 hours after receiving hormone, although the serum sodium concentration decreased from 136.4 to 134.4 m.-eq./l and the serum chloride from 110.2 to 108.4 m.-eq./l there must have been a considerable increase in total m.-eq. of these serum electrolytes when one takes into account the striking dilution of the blood which occurred and which represents a considerable expansion of the volume of extracellular fluid.

It will be also observed (table 2) that on the morning of 1/23/27, or 36 hours following injection of hormone the blood had diluted and returned to approximately normal condition as shown by decrease in hemoglobin, red cell volume and erythrocyte count. The serum potassium was also normal but the serum sodium had risen from 134.4 to 137.4 m.-eq./l and the chloride from 108.4 to 110.2 m.-eq./l. This rise in concentration occurred in the face of 1, further blood dilution and therefore increased volume of extracellular fluid; 2, absence of food intake; 3, renal elimination of both sodium and chloride during the recovery interval.

Study of the renal excretion of sodium and chloride following withdrawal of hormone in this animal clearly shows that no electrolytes were lost from the body since the dog was completely anuric throughout this period. This fact demonstrated that loss of sodium and chloride by way of the kidneys is not a *necessary* accompaniment of adrenal insufficiency any more than increased loss of body water as urine is a *necessary* accompaniment of insufficiency. Since under the conditions of this experiment sodium, chloride and water wastage by the kidneys did not occur, obviously such losses cannot be invoked as an explanation to account for the symptoms of insufficiency in this type of experiment. It will be noted that the usual diuresis and excess renal elimination of potassium occurred during recovery following injection of large doses of hormone.

Our data lead us to conclude that under the special conditions of this

5 grams sodium chloride added to canned dog food (Wilson & Co.) Allowed on 1/24/36 and on 1/25/36

experiment, injections of massive doses of potent cortical hormone mobilize the accumulations of the intracellular water and electrolytes such as sodium, chloride and probably potassium and shift them from intracellular to extracellular compartments and blood stream, thereby bringing about dilution and relief of symptoms.

The cortical hormone employed for injection was isotonic, i.e., it contained small amounts of sodium chloride. It is of interest to calculate the total quantity of salt intake over output (in the urine) during the recovery phase of insufficiency. The dog received no food, the tap water contained negligible amounts of sodium and chloride, hence the only exogenous source of these ions was the cortical extract. A total of 75 cc. of extract containing 9.8 m.-eq. of sodium and chloride was injected. However, during recovery, the dog excreted 10.9 m.-eq. of sodium and 18.3 m.-eq. of chloride. In other words, the animal eliminated more of these electrolytes during recovery then he received and yet the serum concentrations of sodium and chloride increased despite the evident expansion of the extracellular fluid volume.

SUMMARY AND CONCLUSIONS

A. The normal dog with intact adrenals when kept on a salt-free diet for two weeks retains his normal health and vigor. The serum sodium and chloride do not change appreciably but some hemoconcentration may occur.

B. The experimental data obtained from studies on the healthy, vigorous, adrenalectomized dog maintained on a sodium and chloride free diet may be summarized as follows:

1. Urinary loss of sodium, chloride and water are not *necessary* accompaniments of severe adrenal insufficiency.

2. Severe adrenal insufficiency with hemoconcentration, dehydration and circulatory collapse can occur in the absence of significant change in the level of the serum sodium and chloride.

3. Under the conditions of these experiments the serum potassium changes were not significant.

4. The mobilization and redistribution of body fluid which follow hormone injection are evidently associated with a redistribution of electrolytes. The increases in serum sodium and chloride during recovery are, to a large extent, masked by a simultaneous expansion in volume of the extracellular fluid.

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BRAIN POTENTIALS DURING SLEEP

H. BLAKE AND R. W. GERARD

From the Department of Physiology, University of Chicago

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Electrical potentials of the human brain, led from the intact scalp, afford an objective measure of the activity of this organ which may be correlated with other physiological or psychological states. The striking change in neural function between wakefulness and sleep has already been examined with the electrical technique by several workers. Adrian and Yamagiwa (1935) as well as Loomis, Harvey and Hobart (1936) have found the 10/sec. rhythm of the resting wakeful state gone or rare in sleep; when it is replaced by random potentials, or more regular ones at 3–5/sec. (Gibbs, Davis and Lennox, 1935), or brief trains at 14/sec. (Loomis et al.) which wax and wane during a period of a second. The present experiments were likewise directed to a study of the depth of sleep, and to a lesser extent of hypnosis, with the aid of cerebral potentials.

METHODS. 1. *Electrodes.* Copper or silver disc electrodes 1 or 2 cm. in diameter were placed on occiput and forehead. Electrode jelly insured good contact, with the total input resistance regularly under 5000 ohms. Stray potentials from heart, respiratory and eye muscles, etc., were largely eliminated or were controlled.

2. *Recording system.* A four-stage, resistance capacity-coupled amplifier fed a loud speaker, a cathode ray oscillograph, and a crystograph (Offner and Gerard, 1936) or some pair of these. The amplifier reproduced ½ microvolt well above the base line and sine waves at 1/sec. showed a reduction in amplitude of only 10 per cent. At times a variable low-pass filter was inserted in the output to attenuate the higher frequencies, chiefly above 100/sec. Records were taken continuously through the night or at desired intervals.

3. *Measurement of sleep depth.* Since the primary objective of this work was to establish a correlation between depth (or intensity) of sleep and electrical patterns, it was necessary to test the former independently. Sleep intensity is usually measured by the minimal intensity or duration of a stimulus necessary to elicit some predetermined response. Obviously sleep intensity might be modified by each test, so that valid depth curves could be obtained only when 1 or 2 tests were made a night and many data considered statistically. We have ordinarily used 3 or 4 stimuli in one night, which probably have not seriously altered the curve; but even con-

siderable disturbance would not be important since the significant relation is between actual sleep level and potential pattern at the time the test is made. Actually the curve of sleep intensity through the night is in good general agreement with that which Monninghof (1883) and Czerny (1891) obtained under more rigorous conditions and with more extensive material. Further, such other indications of sleep depth as the presence and amount of spontaneous movement, type of breathing, and impressions of the observer agreed with the test stimuli. Finally, since a definite correlation between potential patterns and sleep level did emerge, there is still further assurance that sleep level values were correctly assigned.

The actual procedure, with the subject comfortably in bed, was to ask just audibly, "Are you awake?" The question was repeated at intervals when respiration, movement, response to noise, etc., indicated some change in sleep level. That the question of itself had little influence on the course of sleep is shown by its failure to awaken subjects even in a light stage or to modify the other criteria; and the voice was not above background noise level (a sound-proof room was not used).

As a quantitative test, when some one type of potential wave was clearly present, a note of constant intensity at 1000 cycles a second (on the flat sensitive portion of the auditory acuity curve), obtained from a tuning-fork oscillator amplified into a loud speaker, was sounded. "Are you awake" was repeated at intervals until the subject responded, or until one minute, when the oscillator was stopped. (Auditory adaptation interferes too greatly for longer durations.) Response to a query, rather than some spontaneous signal, was used to indicate awakening since less complete wakefulness is required to obtain it; and often the subject reported in the morning an unbroken night's sleep. It regularly appeared that a second or two before movement no answer to the query was forthcoming while a second or two later the subject replied. As a routine, therefore, the question was asked at 10 second intervals and immediately following the first movement.

The two indices of awakening, visible spontaneous movement and reply to a question, showed consistent agreement with one another. Exceptions occurred in cases of very light sleep, when the response was obtained within 3 or 4 seconds of starting the stimulus without visible movement, and in a very few cases of deep sleep when movement occurred without response. Muscle tone, as measured by potentials led from the gastrocnemius, parallels sleep. During quiet rest volleys appear at brief intervals, which become longer with advancing sleep, until a silent period is reached in deepest slumber. Simultaneous recording of muscle and brain potentials suggests that the fast waves (30/sec.) are closely related to movement and tone—recalling the finding of Jasper and Andrews (1936) that they are centered on the Rolandic region.

Eight men and eleven women in the 20–30 age group served as normal subjects. Twenty-two all-night records were obtained from seven individuals; eight were studied during shorter periods of day-time sleep, and five after voluntary insomnia lasting 36 to 60 hours.

RESULTS. 1. *Normal sleep.* Despite marked individual differences in depth of sleep through the night, the gross picture is reasonably constant. In the first hour or two, sleep is very deep: a full minute of test stimulus elicits no response, the subject is relaxed, breathing is deep and regular, and visible movements occur about once an hour. Following this, sleep becomes lighter and progressively shorter stimuli, down to 5 seconds, cause awakening. By the fourth hour the stimulus is effective in about two seconds, and even without it the subject is restless and moves approximately once in 10 minutes. In the fifth hour sleep sometimes becomes deeper again, the test sound may fail to awaken in 60 seconds, and then recedes until spontaneous awakening in the morning.

This description of sleep depth is only grossly correct since there are swings of considerable magnitude within each period. Our observations are consistent with, but not complete enough to be a confirmation of, the finding (Mullin and Kleitman, unpublished) that a periodic oscillation continues throughout the night, with successive periods showing first higher and then progressively lower maxima but with a final increase in the early morning hours. Further, characteristic sleep patterns, both as to depth and associated potentials, tend to recur in individual persons.

Corresponding to the different sleep levels, not only in time of night but, more convincingly, from moment to moment in the individual case, are distinctive brain potentials. These can be briefly described in terms of the magnitude and regularity of waves at about 30/sec., at 10/sec., and $\frac{1}{2}$ to 3 a sec. and the extent to which one or another dominates the picture.

During wakefulness, the 10/sec. rhythm is present to the extent characteristic of the individual, accompanied by smaller potentials at 20–30/sec. With the onset of sleep both frequencies decrease in amplitude and regularity. As slumber deepens the 10/sec. waves gradually pile up, sometimes with an intermediate stage of improved regularity, and merge to give still slower oscillations; until in deep sleep there remain only regular smooth waves lasting from $\frac{1}{2}$ to 3 seconds. During the genesis, and again later with the decomposition, of these smooth slow waves all transitional stages between them and the 10/sec. rhythm can be observed (fig. 1). When sleep again becomes lighter, during the middle portion of the night, the slow waves melt away and the 10 and 30 a second rhythms reappear fitfully, usually as accompaniments of movements or following a slight sound or other stimulus. In the last third of the night the sequence is sometimes repeated, the slow waves first increasing and dominating the picture and then fading out towards morning, leaving very feeble and irregular potentials like those present in the middle portion.

During deep sleep the test stimulus, which fails to arouse the subject, still modifies the potentials. The slow waves are commonly accentuated and regularized during the first few seconds of stimulation, partially decompose into the 10/sec. rhythm after fifteen, and usually after a minute revert to the original profile (fig. 2). If the stimulus does cause awakening, the 10/sec. waves appear and persist for from 1 or 2 minutes to half an hour. Awakening in response to a stimulus is ordinarily abrupt and accompanied by a similarly sharp appearance of the 10/sec. waves. Spon-

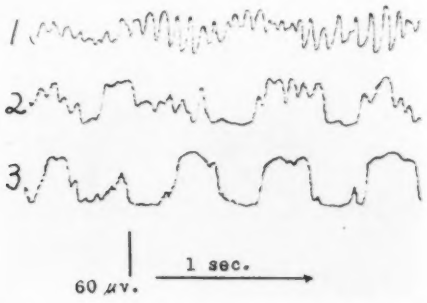

Fig. 1. Crystograph record, medium speed. *1*. Awake, 10/sec. waves marked. Modulation strong. *2*. Moderate sleep, 10/sec. waves as notches on 1/sec. ones. *3*. Deep sleep, 1/sec. waves strong (4+).

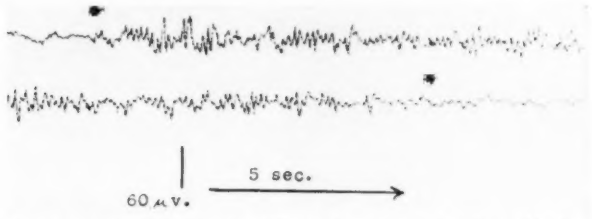

Fig. 2. Crystograph, slow speed. Effect of extraneous noise. Early night, subject falling more deeply asleep. Between stars an aeroplane rapidly approached and receded. Continuous record. Note temporary return of good 10/sec. waves.

taneous waking is more gradual and the sequence is: appearance of slow potentials, first moderately and then quite regular; movement; response to the test question; and, finally, disruption of the slow waves with the appearance of faster ones. In a significant number of cases, however, clear consciousness reappeared before the fast rhythms; and with a subject remaining quiet after waking the rhythm may be absent for five or more minutes—until a voluntary movement or some outside stimulus initiates it.

An analysis of one experiment is presented in table 1, and figure 3 reproduces electrical records corresponding to the categories of the table.

Sample records from another subject are presented in figure 4, and in figure 5 the data for 10 nights are averaged and diagrammed. The correlation between deep sleep and slow regular waves is striking, even through the minor oscillations early in the night, for both appear and disappear

TABLE 1

HOUR A.M.	BEFORE STIMULUS				SECONDS' DURATION OF STIMULUS	AFTER STIMULUS			
	Movement	Answer	1 sec. waves	10 sec. waves		Movement	Answer	1 sec. waves	10 sec. waves
12:15	+	+	−	+++					
12:20	−	−	+++	−					
12:25	−	−	+++	−	60	−	−	+++	−
12:35	−	−	+++	−	15	+	+	−	+++
12:40	−	−	+++	−					
12:45	−	−	++++	−					
12:50	−	−	++++	−	60	−	−	+++	−
1:30	−	−	+++	−					
1:35	−	−	+++	−	Spont. change	+	+	++	Notches
1:43	−	−	++	−	50	+	−	++	Notches
1:55	−	−	++	−	Spont. change	+	+	++	Notches
2:05	−	−	+++	−					
2:15	−	−	++++	−					
2:30	−	−	++	−	Spont. change	+	+	+	Notches
2:40	−	−	−	−					
2:55	−	+	−	−					
3:20	−	−	+	−	Spont. change	+	+	−	++
4:00	−	−	+++	−	15	+	+	−	++
4:10	−	−	+	Notches	Spont. change	+	−	−	++
4:30	−	−	+	Notches					
5:00	−	−	+++	−					
5:50	−	−	+	Notches					
6:00	+	+	−	++					

− denotes complete absence of phenomenon.
+ denotes occasional waves. For other phenomena it denotes presence.
++ denotes poorly repeated wave trains which are not marked, regular or maintained.
+++ denotes good regular continued wave trains.
++++ denotes extremely regular continued wave trains.
"Notches" denotes 10/sec. waves appearing as notches superimposed on 1/sec. waves.
"Spont. change" denotes spontaneous change in potentials observed.

together (fig. 6). Hardly less regular is the appearance of the 10/sec. or faster rhythms immediately following a movement or a disturbing stimulus and their practical absence at all other times. Occasional exceptions occur, however, especially in that feeble irregular potentials may be present and yet the test sound evoke no response in 60 seconds. The later subjec-

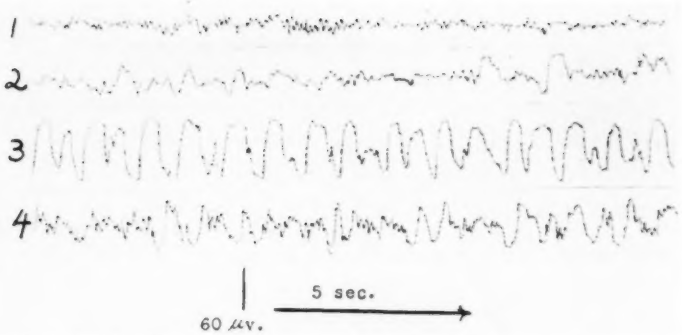

Fig. 3. *1.* Awake, 10/sec. waves good. *2.* Light sleep, 1/sec. waves at intervals (2+). *3.* Deep sleep, 1/sec. waves strong (4+). *4.* Moderate sleep, 10/sec. waves as notches on 1/sec. ones.

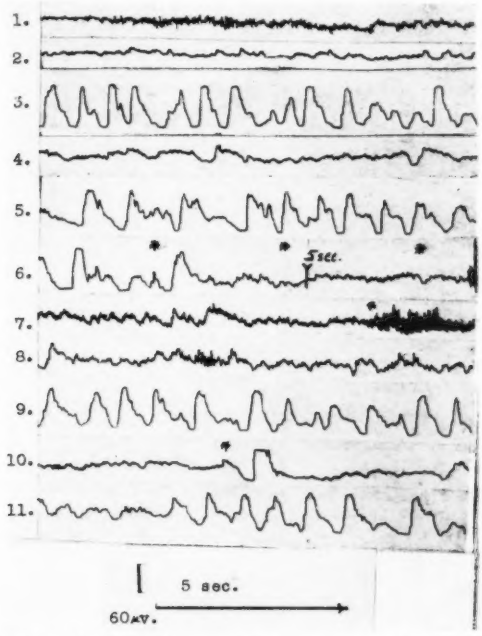

Fig. 4. *1.* 12:00 m. Awake 10/sec. and 30/sec. waves. *2.* 12:30 a.m. Drowsing; No. 30/sec., feeble 10/sec., suggestion of 1/sec. *3.* 1:10. Deeply asleep 1/sec. waves marked; at 1:11 awakening by hummer. *4.* 1:13. Falling asleep; 10/sec. waves dominant. *5.* 1:25. Fairly deeply asleep; 1/sec. waves good. *6.* 1:26. From sleep to wake—sound stimulus on during length of record. No response to questions at star. Awake and moves at end. *7.* 1:26½. Drowsy 30/sec. waves marked, actual small movement at star. *8.* 1:30. Fair sleep; 10/sec. waves present; good spindle occurs. *9.* 1:40. Deep sleep; 1/sec. waves present, good spindle occurs. *10.* 2:00. Fair sleep. No response to question at star but wave follows. Irregular 10/sec. *11.* 2:30. Fairly deep sleep 10/sec. waves passing into good 1/sec. ones.

tive reports then indicate greater wakefulness than had been shown by the objective tests.

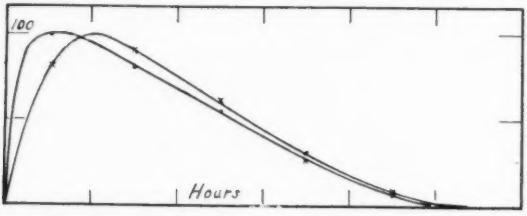

Fig. 5. Depth of sleep *x-x-x*, and prominence of slow waves *o-o-o* for first 6 hours of sleep. Average curves for 10 nights on five subjects. Sleep depth ordinates, duration of sound to awaken. Slow wave ordinates obtained by assigning 0 to 4+ (see table 1) for each five minute period and averaging for the hour. Arbitrarily plotted to same maximum as sleep depth curve. Abscissa, time in hours.

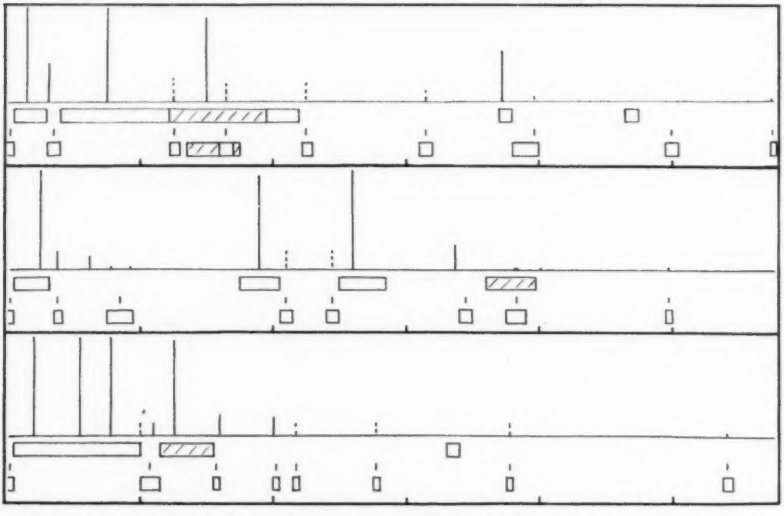

Fig. 6. Records of three single nights. Time in hours. Upper graph: Sleep depth, the height of the line indicates duration of stimulus required to elicit a response. Dotted lines estimated from movement, etc. Middle graph: Blocks represent 1/sec. waves. Cross-hatching indicates poor waves (1 or 2+). Lower graph: Blocks represent 10/sec. waves; cross-hatching indicates poor waves. Movements as vertical dashes between 1/sec. and 10/sec. blocks.

2. *Modified sleep.* In unconditioned subjects, daytime napping is ordinarily much lighter than the conditioned night sleep. Increase and decrease in intensity are more gradual and in very few cases was sleep deep enough for the slow potentials to appear. One seeming difference from

night sleep is in the 10/sec. rhythm which is often present and even exaggerated at sleep levels which, during the night, are associated with the absence of all regular rhythms.

In sharp contrast to napping are the results following prolonged voluntary insomnia, in 3 cases lasting 60 hours. A subject in this condition, sitting quietly awake at any time of the day or night, shows the characteristic waking potentials. Simply relaxing precipitates him into a sleep of maximal intensity. The brain potentials similarly show a prompt change to very large, regular and slow waves; and forcible awakening restores the

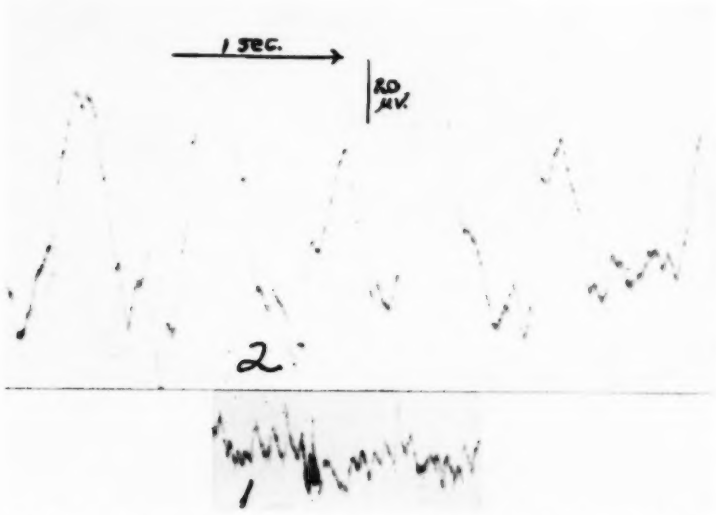

Fig. 7. Cathode ray record. Subject after 60 hours' insomnia. *1*. Awake. *2*. Forty seconds later, deeply asleep.

original potential pattern. It is, in fact, possible to pass from 10/sec. to 1/sec. and back to 10/sec. waves within a minute by allowing the subject to sink into sleep and reawakening him (fig. 7). Further, when the subject is allowed to sleep undisturbed, after an insomnia period, the regular slow waves last 3–4 hours instead of their usual 2.[1]

3. *Hypnosis.* One subject was repeatedly brought into a deep cataleptic stage of hypnosis although never fully into the somnambulistic one. In this young man a good 10/sec. rhythm was normally present and persisted,

[1] Kleitman has recently found benzidrene administration to counteract the effects of prolonged insomnia. We find, similarly, that it diminishes the potential pattern of deep sleep which appears after an insomnia period.

sometimes with diminished amplitude, throughout the hypnotic period. A 14/sec. rhythm was observed but the very slow waves of sleep were never definitely indicated. It proved possible, as reported also by Loomis, Harvey and Hobart (1935), to abolish the 10/sec. rhythm by suggesting a light shining in the eyes although actually none was present. The reverse experiment was not successful at this level of hypnosis, for actually shining the light while suggesting darkness in all cases abolished the rhythm. The same results were obtained whether the eyes were open or shut. Other suggested stimuli were without influence on the brain potentials, except that often with each assertion, "You are falling deeper asleep" the 10/sec. rhythm became more distinct.

Discussion. The primary conclusion permitted by these findings is that a definite parallelism exists between the type of brain potentials present and the stage of sleep or wakefulness. This is usually (but not always) so exact that from the appearance of the potential record it is possible to predict the amount of stimulus which will be required to awaken the sleeper.

Qualitatively, these potential patterns are closer to the findings reported by Gibbs, Davis and Lennox (1935) than those of Berger (1935), Adrian and Yamagiwa (1935) or Loomis, Harvey and Hobart (1936). The three latter groups of observers have emphasized the diminution of the 10/sec. rhythm without remarking the large, slow rhythm which replaces it. Even in the work of Gibbs et al. frequencies of 3–5/sec. have been emphasized rather than the $\frac{1}{2}$–3/sec. rhythm which we have found in deep sleep. Such waves are seen, however, in some of their records. Possibly in following subjects through the entire night we have dealt with deeper sleep than did earlier experimenters; or the slow waves may have been outside the amplifier range of some and so lost.

Although the slowest rhythm seems to be determined mainly by sleep depth, it is our impression that the 10/sec. and faster rates depend on at least two other factors in addition to actual sleep intensity. For one thing, when the subject reaches a certain level in the course of increasing sleep-depth, these waves are often prominent, whereas when this same level is attained in passing from deep sleep towards wakefulness they may be nearly or entirely absent. The immediate past is therefore significant, presumably by virtue of the pre-existing excitation level. The second factor is related to muscle tone and movement, for the faster rhythms commonly appear in association with muscle contractions.

We can consider the 10/sec. rhythm as an index of the activity of brain cells relatively undisturbed by current transient stimulation yet, as the result of previous excitation, continuing their rhythmic activity. As their thresholds rise with continued absence of impinging impulses, that is,

as the facilitation produced by previous ones wears off and excitatory state decreases, the regular 10/sec. beat of the cerebral neurones gradually disappears. Inflow of new impulses, if not too intense, reëstablishes this rhythm; still stronger stimulation again disrupts it. On this view, the absence of the 10/sec. rhythm in or following deep sleep is due to failure of the electrical beat of the individual cells while its absence in ordinary wakefulness, with open eyes, body movements and directed attention, is due to asynchrony of the many beating cells. This interpretation and the evidence from many directions that favors it have been discussed at length elsewhere and need not be repeated (Gerard, 1936; Adrian, 1936; Bremer, 1936).

It remains to account for the striking slow rhythm of deep sleep. The change from a regular rhythm at 10/sec. to one at 1/sec. can hardly be due to an improved synchrony, for the regularity in rate and amplitude of the faster rhythm is evidence that excellent unison is already present at this frequency. Either some cells with a faster natural frequency of beat become inactive in sleep while slower ones become active, or the same cells markedly slow their beat. There is no basis for the more complex assumption involved in the first alternative while the second is quite in harmony with the interpretation already advanced for the 10/sec. rhythm. In this case, too, weak stimulation facilitates the rhythm whereas strong stimuli abolish it.

Let us assume that individual neurones beat at a rate of 10/sec. when their level of excitation is above some critical value but fall to a slower rhythm when the excitation level is lower. This seems highly probable since stronger excitation does give higher frequencies for natural stimulation of receptors (Adrian, 1932) or motor cells (Adrian and Bronk, 1929), or for electrical stimulation of muscle or nerve (Fessard, 1936; Katz, 1936; Erlanger and Blair, 1936; Gasser and Grundfest, 1936). The limits are complete inactivity, on the one hand, and, on the other, a frequency set by some recovery process in the cell (related to, but not necessarily the same as, the refractory period for repeated discharge of impulses). What is less clear is whether the change of frequency is continuous or by steps. The latter case is exemplified, for example, in the fall of nerve responses from one to each stimulus, to one to two or three or more, as the frequency of stimulation steadily increases (Derbyshire and Davis, 1935) or as the nerve progressively equilibrates to fixed stimuli (Gerard and Forbes, 1928). The present results are suggestive of a similar situation. The 10/sec. rhythm does not become progressively slower but rather continues as smaller and smaller ripples on an increasing slow wave, until indistinguishable; as would occur if one cell after another dropped abruptly from the fast to the slow rate. We cannot, however, insist on this interpretation

since, in long complex records, almost any potential profile can be found. In fact even the slow waves show frequencies ranging from 3 to ½ a second or less and it is not established that the shifts occur by steps.

It remains to consider the period of feeble irregular potentials that intervenes between the disappearance of the regular slow waves of deep sleep and the reappearance of the regular faster ones on waking or movement. Again, by analogy with the better studied 10/sec. rhythm, this may be regarded as a stage in which the cells are still beating at the slow rate but have become asynchronous under a mild excitation not sufficient to start a faster beat. The alternate view is not excluded, however,—that even the slow beat has petered out: which implies that following deep sleep the brain cells may be relatively free from stimulation yet highly irritable. This could occur if afferent connections become inactive during sleep, permitting the activity of cortical neurones to run down—as is indicated by the absence of discharges, while the rest period permits greater irritability to build up; then even feeble stimuli could reinitiate activity of the cells and also wake up the sleeper. In any event, afferent stimulation at this time commonly does first improve the slow rhythm and then disrupts it in favor of the 10/sec. one.

The findings during hypnosis throw additional light on the problem of the control of neurones by impinging nerve impulses. Since the suggestion of light, via auditory channels, can disrupt the regular beat of cells in the visual cortex it is not essential that the nerve impulses reach these cells along direct optic paths. Presumably indirect connections from the frontal lobes, under conditions of "visual attention," can exert the same control over the occipital neurones as can the fibers of cells in the geniculate body.

SUMMARY

Brain potentials of young adults were studied during normal night sleep, afternoon napping, post-insomnia sleep and, in one case, hypnosis.

Depth of sleep was independently determined by the duration of a fixed sound required to awaken the subject (response to a question), by movement, respiration, etc.; and an orthodox sleep-depth curve for the night so obtained.

Potential patterns correlate with sleep depth not only over long time changes but, in most cases, rather accurately over short time swings.

Deep sleep is regularly associated with a large regular potential wave at a frequency of ½ to 3 a second; lighter sleep with feeble irregular potentials or with the 10 a second rhythm. Further details on transition potentials from light to deeper sleep and especially during spontaneous or stimulated awakening are described.

The results are interpreted in terms of excitation levels of cerebral

neurones and of the play of nerve impulses upon them. With low excitation a slow rhythm is manifest, with stronger excitation a faster one, and in transition between these states asynchrony prevents the manifestation of any regular beat.

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SUPERFECUNDITY[1] IN RATS TREATED WITH MARE GONADOTROPIC HORMONE

H. H. COLE

From the College of Agriculture, University of California, Davis

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Superovulation in mammals treated with various gonadotropic preparations has been amply demonstrated (Smith and Engle, 1927; Leonard and Smith, 1933; Hertz and Hisaw, 1934; Foster and Hisaw, 1935; Evans and Simpson, 1935 and Cole, 1936). Engle (1927) appears to have been the only one reporting on superfecundity of mammals treated with gonadotropic substances. He obtained as many as 29 implanted embryos on the 9th day of pregnancy in mature mice receiving pituitary transplants. No unusually large litters were delivered, however.

Because of the ease of producing superovulation in immature rats this species was used in the present experiment. Initially the mature rat, rather than the immature, was selected as having greater potential capacity to nourish a large number of fetuses; although treated immature rats are known to produce living young (Cole, 1936), contrary to the findings of Mirskaia and Crew (1930) and of Engle (1931) working with mice, yet the small number born did not justify high expectation of success in the immature animal. After failure appreciably to influence fecundity in the mature rat, however, the immature animal was again resorted to, with marked success. The small number of young born to the immature rats

[1] The term "superfecundity" is here being used to denote the ability to implant an unusually large number of fetuses. The use of "fecundity" is by no means uniform; for example, Pearl and Surface (1909) propose, "We would suggest that the term 'fecundity' be used only to designate the innate potential reproductive capacity of the individual organism, as denoted by its ability to form and separate from the body mature germ cells. Fertility in the female will depend upon the production of ova and in the male upon the production of spermatozoa." There are some objections to the usage suggested by Pearl and Surface. First, the derivation of the word intimates its use in reference to fetation. Next, ovulation is a well-defined word in common use to denote the liberation of ova from the ovary and fertility is used to designate the ability to produce mature germ cells which can impregnate and bring forth living offspring. Finally, fecundity is commonly used in reference to the ability of an animal to give birth to a large number of offspring but the word "prolificacy" describes this characteristic accurately. It would appear desirable, therefore, to use the term "fecundity" in reference to mammals to denote the ability to implant.

in the earlier experiments was found to be related to the small amount of hormone injected. This paper deals, therefore, with the effect of various dosages of mare gonadotropic hormone upon the fecundity of mature and immature rats.

The rats used are from the Long-Evans strain. Under the conditions in our colony the average date of opening of the vagina in 100 rats is 43.6 days, with a range of 33 to 55 days (Blunn, unpublished). As Long and Evans (1922) have pointed out, the opening of the vagina is closely associated with sexual maturity. The average litter size for 243 litters in our breeding colony is 11.5, with a range from 1 to 19. Only 2 litters of 19 have been encountered in several thousand.

To avoid discrepancies due to inaccuracy of assay methods, a single batch of serum was used for most of the experiments. It contained 50 rat units per cubic centimeter, tested according to the method of Cole, Guilbert and Goss (1932). This serum was run through a Berkefeld filter, treated with merthiolate, and stored at 34 to 36° F. Serum so treated will not be reduced in potency more than 50 per cent over a five-year period.

The effect of mare gonadotropic hormone on the mature female rat. Fecundity. The results obtained by injecting the hormone into mature female rats are summarized in table 1. Single injections were given excepting in one group receiving small daily injections over a 15-day period. Those injected in dioestrum were bred in the following oestrus about 2 days after injection; those in oestrus in the succeeding oestrus about 4 days after injection; and those in metoestrum about 3 days after injection. A typical protocol of an animal injected in metoestrum follows:

Rat B 9680
10/31/35—small epithelial cells (prooestrum)
11/ 1/35—cornified cells
11/ 2/35—leucocytes, small epithelial and cornified cells (metoestrum); 12 rat units mare gonadotropic hormone subcutaneously
11/ 4/35—Small epithelial cells (prooestrum) placed with ♂ G 8886
11/ 5/35—sperm
11/18/35—R. B. C. in smear (placental sign)
11/26/35—litter of 17 (9♀ and 8♂).

Approximately 50 per cent of the pregnant rats were sacrificed on the 12th to 14th day of pregnancy to ascertain the number of implanted fetuses. The remainder were allowed to go through pregnancy. These two procedures were followed to determine whether or not more fetuses were implanted than were born. Actually very little difference was found. The average number of implanted fetuses for rats receiving 12 rat units in metoestrum, for example, was 13.6, whereas the average number of young born for rats given a similar treatment was 13.4. These two groups, consequently, were united for the data in table 1.

The data indicate that both the time of injection and the amount administered affect fecundity. First we will consider how these factors influence the number of fertile matings. Though we do not have complete data on the number of nonfertile matings for noninjected controls, it is certainly less than 10 per cent.[2] As table 1 shows, 70 per cent of the matings following the administration of 12 rat units in dioestrum were infertile as compared with 27 per cent for those receiving 12 rat units in metoestrum. In other words, apparently fertility is less when a given amount of hormone is injected in dioestrum than when a similar amount is injected during metoestrum. Smaller amounts do not have this adverse effect.

TABLE 1

The effect of mare gonadotropic hormone on the fecundity of the mature female rat

STAGE OF CYCLE WHEN TREATED	AMOUNT INJECTED	NUMBER OF RATS WHICH MATED	PER CENT NONFERTILE MATINGS	NUMBER OF RATS PREGNANT	AVERAGE LITTER SIZE	PER CENT PREGNANT WITH 15 OR MORE IN LITTER
	rat units					
Dioestrum	8	7	14	6	12.7	16.7
	12	26	70	8	9.3	12.5
Oestrus	8	10	20	8	9.0	0
Metoestrum	3 to 8	22	5	21	10.9	9.5
	12	44	27	32	13.5	46.9
	18 to 24	14	80	3	9.3	0
Daily injections on 15 days	¼ daily	8	0	8	11.4	0
	½ daily	8	0	8	10.0	0
	1 daily	6	0	6	9.5	0
	Controls	Data incomplete	Data incomplete	243	11.5	9.0

Next let us consider dosage. With increasing dosage the number of fertile matings decreases. This fact is particularly well shown in the group injected during metoestrum—only 5 per cent nonfertile matings for those receiving 3 to 8 rat units, 27 per cent for those receiving 12, and 80 per cent for those receiving 18 to 24. These figures are in accord with data on the immature rat showing that excessive dosage inhibits ovulation.

We will next consider the effect on litter size. The injection of 12 rat units in metoestrum definitely increased the average litter size (table 1).

[2] Evans and Burr (1927) reported 82 to 95 per cent implantations in 1500 positive matings of rats on natural foods.

Nearly half the rats had 15 or more fetuses; 7 out of 32 had 17 or more; and 2 had 19 young. As stated above only 2, out of several thousand, litters of 19 have appeared in our breeding colony. The litter size in rats receiving treatment other than 12 rat units in metoestrum did not vary from the normal sufficiently to have statistical significance. Perhaps this fact would indicate that only in metoestrum in the rat can injected gonadotropic hormone give an additive effect to that of the secretion of the animal's own pituitary. Perhaps, then, metoestrum is the time of increased gonadotropic-hormone secretion in this species. In the mouse, Engle (1927) increased the number of implanted fetuses by beginning the treatment on the first day of dioestrum.

An earlier paper showed that superovulation could be induced with 16 rat units of mare gonadotropic hormone. Later in this paper we will show that half this amount is more satisfactory, as superovulation is more regularly induced with the smaller dosage. Considering an increased activity of the pituitary of the mature animal over that of the immature, smaller amounts would apparently be necessary to produce superovulation. As 12 rat units gave definitely better results than 8, however, this is not the case. At no time can we refer to superovulation in the mature rat, for the number of young does not exceed the number that occur normally. All that has been done is to increase the percentage of large litters.

The question arises as to why we have not been able to increase litter size more markedly in the mature animal. Is it because superovulation is not induced or because the fertilized ova are not implanted? To obtain data on this point, 9 rats injected with 12 rat units in metoestrum and bred in the succeeding oestrus were sacrificed, the oviducts sectioned serially, and every fourth section saved in order to count the number of ova liberated. Then 7 non-injected controls were treated similarly. Because some of the fertilized ova were in the two-cell stage, a difficulty arose. The oviducts were imbedded in paraffin, sectioned, and stained with hematoxylin and eosin. Unaccountably, the zona pellucida does not show; and inasmuch as only every fourth section was saved it sometimes becomes difficult to distinguish between a single segmented ovum (in which the two cells are not in direct contact at the point of section) and two separate ova. Thus the absolute counts of ova are not reliable. Nevertheless, the results in the injected group can be compared with those in the control group. In the noninjected group the average number of ova is 18.9, with extremes of 11 and 24. In the injected group the average is 20.5, with extremes of 13 and 24. Almost certainly, therefore, the failure greatly to increase litter size in the mature animal results from the inability of mare gonadotropic hormone to induce superovulation.

Length of cycle. Although only indirectly related to the problem at hand, the effect of the hormone on the length of the cycle is interesting.

The average length of 92 cycles in control animals was found to be 4.48 days, as compared with 3.91 days for 72 cycles in animals injected in metoestrum. Thus the cycle is definitely shorter in the injected group. Only 3 cycles longer than 4 days were encountered in the injected group, whereas there were 38 in the control group.

The effect of mare gonadotropic hormone on the fecundity of the immature rat. At the time of injection the rats used were 26 to 31 days of age. Most of them were 28 days old. As stated above, females in our colony reach maturity between the 33rd and 55th day, with an average of 43.6 days. To determine the youngest age at which conception would result in the rat after injection of gonadotropic hormone, 12 rats were injected with 3 and 16 with 6 rat units on the 22nd day of age. Two days later they were placed with mature males. None receiving the larger dose mated, whereas 4 of 12 receiving 3 rat units had bred by the 72nd hour after injection. Of the 4, 2 had 6 and 7 normal-appearing fetuses respectively when sacrificed 12 days after mating. Rats injected before the 21st day do not ovulate with any regularity. Thus mating and the consequent implantation of fertilized ova will occur at the earliest age at which ovulation can be regularly induced in the rat.

The procedure followed was to inject the rats in the morning; 60 hours later each female was placed in an individual cage with a mature male. Practically all those that mated did so by the following morning—that is, by the 72nd hour after the injection. Males of various ages were employed to determine whether mating occurred more frequently when the size of the male more nearly approximated that of the female. Old males, however, weighing 700 grams (eight to ten times the weight of the immature females) proved just as satisfactory as younger males. Some of these rats were sacrificed 10 to 12 days after mating, others at 17 to 22, while another group was maintained until the end of pregnancy. The results are summarized in table 2.

The number of implanted fetuses in these immature females is extraordinary—as many as 28. The *average* number implanted approximates the largest number found in the mature animal. Figure 1 shows a uterus on the 12th day with 25 fetuses; figure 2 a uterus containing 26 entirely normal-appearing fetuses on the 17th day of pregnancy. Quite remarkably, the burden placed on these youthful rats is carried with no sacrifice, but rather with an apparent increase in body growth as determined either by weight (after removal of the uterus and its contents) or by body-length measurement. In figure 3 the uterine horns, though left attached to the cervix, have been unfolded and the suspensory ligaments severed to illustrate the development of the uterus just before term.

As data in table 2 show, 49 per cent of the immature rats placed with

males mated. As in the mature rat, the percentage of fertile matings is inversely proportional to the amount of hormone administered. Ovulation occurs less regularly with the larger doses. By comparing rats autopsied at different stages of pregnancy one can see that considerable resorp-

TABLE 2

Data on the fecundity of immature rats given a single injection of mare gonadotropic hormone, bred 3 days later and sacrificed at various times after mating

The rats were 26 to 31 days of age at the time of the injection

DAYS AFTER MATING WHEN SACRIFICED	AMOUNT INJECTED	NUMBER OF RATS INJECTED	NUMBER OF RATS WHICH MATED	NUMBER OF RATS PREGNANT	AVERAGE NUMBER OF NORMAL FETUSES	AVERAGE NUMBER OF RESORBING FETUSES	TOTAL AV. NUMBER OF FETUSES	PER CENT WITH 17 OR MORE FETUSES	NUMBER OF FETUSES IN INDIVIDUAL CASES
	rat units								
10 to 12	6 to 10	120	51	36	14 5	2 8	17 3	69 4	2, 2, 3, 5, 7, 10, 12, 13, 14, 15, 16, 17, 17, 17, 17, 17, 18, 18, 19, 19, 19, 19, 19, 20, 21, 21, 22, 23, 23, 23, 23, 24, 25, 25, 27, 28
	12 to 16	55	23	6	14 5	4 7	19 2	66 7	1, 16, 23, 24, 25, 26
17 to 22	8 to 10	81	41	30	12 8	3 0	15 8	43 3	1, 6, 7, 9, 10, 12, 12, 12, 13, 13, 13, 15, 15, 15, 16, 16, 16, 17, 17, 18, 18, 19, 20, 20, 22, 22, 23, 24, 26, 27
	16	17	12	2	10 5	8 5	19 0	100 0	17, 21
Allowed to go through pregnancy	8	15	14	14	12 6†	?	12 6	21 4	7*, 7, 7, 10*, 11*, 11*, 14*, 14*, 14*, 14*, 15, 17*, 17*, 18

* All rats in litter were alive at birth.

† An average of one dead rat per litter was found. Others may have been eaten.

tion occurs. There is little question that larger litters would be born than are recorded if a larger population were employed, for as many as 23 living fetuses have been encountered on the 21st day of pregnancy. No doubt some expelled, resorbing and dead fetuses were devoured by the mother

before being observed. The average weight of these young at birth was 5.5 grams as compared with 6.4 grams for first-litter young in our breeding colony.[3]

Discussion. The question of primary interest appears to be not why such an unusual number of fetuses are implanted in the immature rat but rather why this phenomenon does not occur in the mature animal similarly treated.[4] Judging from the data, the root of the difficulty lies in the inability to liberate greatly excessive number of ova in the mature animal by

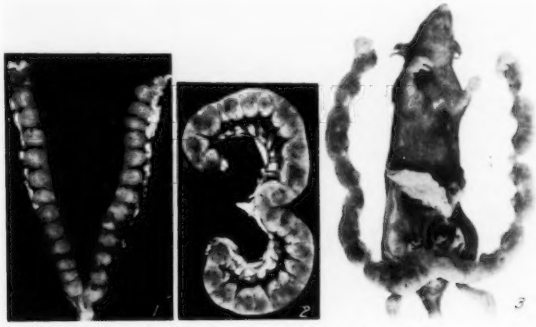

Fig. 1. Uterus of rat B 2391 sacrificed on the 12th day of pregnancy with 25 fetuses.

Fig. 2. Uterus of rat B 2443 sacrificed on the 17th day with 26 fetuses. On opening this uterus, the fetuses were found to be uniform in size and apparently normal.

Fig. 3. Rat G 2932 sacrificed on the 21st day of pregnancy. The uterine horns were left attached to the cervix uteri to show the development of the uterus in comparison with the rat. Twenty small but otherwise apparently normal fetuses are present. In addition to these there are three badly macerated embryos at the points where relatively large distances between fetuses occur.

the methods employed. Superovulation has been induced in mature females of other species. Smith and Engle (1927) and Engle (1927) induced

[3] Engle (1931) did not obtain any abnormally large litters in mature mice treated with pituitary transplants even though an excessive number of embryos were implanted. He attributed this to resorption due to overcrowding. Considering the potential capacity of the uterus to develop, as evidenced by the ability of the immature rat uterus to nourish more than 20 fetuses, hormone deficiency of some kind, in my opinion, is a more likely explanation.

[4] Davenport (1920) presents evidence on human twinning which indicates that, ". . . families which readily produce twins do so not only because in the mother the eggs were laid in pairs, but also because in the father the sperm is active, abundant and without lethal factors, so that the number of eggs fertilized and brought to full term approaches a maximum." Lush (1925) submitted similar data in regard to twinning in cattle. Though the same group of males was not used in both the immature and mature females in the present experiment, all of the males were sufficiently closely related to make it appear very unlikely that they exercised any influence on the differences obtained in the two age groups.

it in the mouse with pituitary implants; Foster and Hisaw (1935) did the same in anoestrous cats by the subcutaneous injection of follicle-stimulating hormone followed by the intravenous injection of another pituitary fraction, the "luteinizing" hormone. We now have considerable data on the effect of mare gonadotropic hormone on the mature female of three species—the rat, the sheep and the rabbit. In none of these has clear-cut evidence of superovulation been obtained. Were it not for the evidence cited above of superovulation in other mature forms, one might assume that the mature animal has some defence mechanism controlling the number of eggs liberated which the immature animal lacks. Corpora lutea cannot be concerned—in all instances at least—for we have treated ewes during anoestrum, when corpora are absent, with no evidence of superovulation.

Hisaw and his associates emphasize the importance of the route of administration and the correct balance of two gonadotropic hormones in producing ovulation. Since the subcutaneous route was used throughout the present study, the route of administration cannot be used in explaining the discrepancy between the immature and mature rat. The balance of two hormones cannot be so easily disposed of, however, for we must consider not only the material injected but also the pituitary secretions of the injected animal. Possibly the pituitaries of the two age groups may be secreting different proportions of two gonadotropic hormones; but this is only one of many plausible explanations that might be advanced. Interestingly, superovulation, though produced in immature animals by gonadotropic substances from several sources, has been induced in mature animals only by pituitary transplants or extracts.

SUMMARY AND CONCLUSIONS

The effect of mare gonadotropic hormone on fecundity was determined in 155 instances in mature animals and in 288 immature animals. Only in the immature animal did the injections result in superovulation and in the implantation of a number of fetuses far exceeding that found in the normal mature female.

Mature rats were injected with amounts ranging from 3 to 24 rat units in dioestrum, oestrus, and metoestrum. The average litter size and the percentage of large litters were definitely increased by injecting 12 rat units in metoestrum; but no litter exceeded in size the largest found in control animals. Excessive doses reduced the percentage of fertile matings in both mature and immature rats.

Implantation of fetuses was found to occur in rats injected on the 22nd day and mated on the 25th day of age. Of rats injected on the 26th to 31st day of age 49 per cent mated. Necropsy on the 10th to 12th day of pregnancy revealed an astonishing number of implanted fetuses—as many

as 28. Of the rats sacrificed at this time, 38 per cent had in excess of 20 fetuses. One rat sacrificed on the 17th day had 26 normal-appearing fetuses; and another on the 21st day had 23 living fetuses. In a rather small series the largest number born was 17 living young.

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THE REMOVAL OF INTRAVENOUSLY INJECTED BILIRUBIN FROM THE BLOOD STREAM IN THE DOG

CARL A. DRAGSTEDT AND MOORE A. MILLS

From the Department of Physiology and Pharmacology and the Department of Pathology, Northwestern University Medical School, Chicago

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There are a number of observations which indicate that intravenously injected bromsulphalein is removed from the blood stream to a large extent by the littoral cells of the reticulo-endothelial system and that a blockade of these cells with India ink or saccharated iron oxide causes a marked retention of the dye (1, 2). We have also reported (3) that the intravenous injection of bilirubin causes a similar though less marked retention of dye. This might be interpreted as indicating that a bilirubinemia produces a blockade of the reticulo-endothelial system or at least that these cells are concerned in the disposal of injected bilirubin. As a result of the pioneer work of von Bergmann (4) and Eilbott (5) the employment of intravenously injected bilirubin as a clinical test of hepatic function has attained considerable importance. It is the object of the present paper to study the removal of intravenously injected bilirubin from the blood stream under a variety of experimental conditions in an attempt to establish the relative rôles played by the reticulo-endothelial system and the hepatic parenchymal cells.

METHODS. Adult, mongrel dogs of medium size were used. Pure bilirubin (Eastman Kodak Company) dissolved in distilled water with the aid of a few drops of 10 per cent sodium hydroxide solution was injected intravenously in doses of 5 and 10 mgm. per kilo. These doses, which are relatively much larger than those employed clinically, were chosen so that the experimental error involved in the quantitative plasma bilirubin determinations would be minimized. Blood samples (oxalated) were withdrawn at 5, 30, 60, 90 and 120 minutes from either the femoral or external jugular vein. The bilirubin determinations were made on the centrifuged plasma by the Gibson and Goodrich (6) modification of the Van den Bergh procedure. Operative procedures were performed aseptically under morphine-ether anesthesia. The rate of removal of bilirubin was determined in normal animals, in animals under ether-barbital anesthesia, after reticulo-endothelial blockade, and after partial and complete biliary obstruction. Attempts to influence the removal of bilirubin under

the above conditions by the administration of decholin as a cholagogue were also studied.

RESULTS. 1. *Bilirubin removal in unanesthetized dogs.* The data on 9 normal dogs are shown in table 1. Bilirubin is removed from the blood stream in dogs evidently much faster than in man (7). With the 5 mgm. per kilo dose the bilirubin is practically completely gone in 60 minutes, and after the 10 mgm. dose the plasma is nearly free of bilirubin in 90 minutes. It should be noted, however, that approximately 60 per cent of the injected bilirubin is unaccounted for in the 5 minute sample. For example, if we assume blood volumes of 10 per cent of the body weight and plasma volumes of 5 per cent of the body weight, the injection of 5 mgm. per kilo of bilirubin should give an initial theoretical concentration of 10 mgm. per 100 cc. and the injection of 10 mgm. per kilo should give

TABLE 1

Bilirubin clearance in normal dogs

EXPERIMENT NUMBER	INITIAL PLASMA BILIRUBIN	BILIRUBIN INJECTED	PLASMA BILIRUBIN IN MGM. PER 100 CC.				
			5 minutes	30 minutes	60 minutes	90 minutes	120 minutes
		mgm./kilo					
12	0	5	4.3	1.5	Tr.	0	0
13	0	5	3.3	1.6	Tr.	0	0
18	0	5	3.8	1.2	0	0	0
20	0	5	3.5	0.5	0	0	0
47	0	5	5.2	1.5	Tr.	0	0
48	0	5	4.3	1.8	0	0	0
19	0	10	7.2	2.0	0.5	0	0
21	0	10	8.7	3.5	0.5	0	0
22	0	10	8.7	4.8	3.0	0.5	0

an initial concentration of 20 mgm. per 100 cc. According to Takane (8) the injection of pure bilirubin does not increase the output of bile so that the greater part of this initial drop cannot be explained by hepatic excretion. Also according to Eilbott (5) it cannot be explained by adsorption to circulating blood elements. The question of this initial rapid removal of injected bilirubin will be referred to later.

2. *Bilirubin removal in dogs during anesthesia.* The data on 4 dogs under ether-barbital anesthesia are shown in table 2. The dogs received approximately 0.3 gram of sodium barbital per kilo after a preliminary ether induction. The 5 minute plasma samples show a slightly higher concentration of bilirubin than in normal animals and a definite prolongation of the time for complete removal. Bilirubin was still present in the 2 hour samples of plasma even with the 5 mgm. per kilo dose. That there is a decrease in the rate of bile excretion during anesthesia is well

known (8). The delay in removal of bilirubin from the blood during anesthesia may be theoretically accounted for by this decrease in bile flow although no definite evidence upon this point was obtained.

3. *Bilirubin removal in dogs after ligation of the bile ducts.* The data on 6 dogs after ligation of various types are shown in table 3. In two animals (expts. 30 and 31) the bilirubin was injected immediately after the ligation of the common and cystic ducts and therefore while the ani-

TABLE 2

Bilirubin clearance in dogs during anesthesia

EXPERIMENT NUMBER	INITIAL PLASMA BILIRUBIN	BILIRUBIN INJECTED	PLASMA BILIRUBIN IN MGM. PER 100 CC.				
			5 minutes	30 minutes	60 minutes	90 minutes	120 minutes
		mgm./kilo					
32	0	5	5.5	2.4	2.2	1.4	0.8
33	0	5	5.7	2.4	1.7	0.8	Tr.
16	0	10	9.1	6.8	6.4	8.0	7.0
46	0	10	9.1	6.0	4.2	1.9	1.9

TABLE 3

Bilirubin clearance in dogs after ligation of bile ducts

EXPERIMENT NUMBER	INITIAL PLASMA BILIRUBIN	BILIRUBIN INJECTED	PLASMA BILIRUBIN IM MGM. PER 100 CC.					REMARKS
			5 minutes	30 minutes	60 minutes	90 minutes	120 minutes	
		mgm./kilo						
26	2.0	10	11.7	14.3	12.5	13.3	11.7	Common D. Lig. 24 hours
30	0	5	4.0	5.0	4.5	5.5	5.5	Common and Cystic D. Lig. 1 hour
31	0	5	3.8	3.5	4.5	4.5	3.8	Common and Cystic D. Lig. 1 hour
44	4.5	5	10.0	10.0	11.2	10.4	10.0	Common and Cystic D. Lig. 72 hours
45	5.8	5	10.0	8.3	10.2	9.8	10.0	Common and Cystic D. Lig. 72 hours
25	0	10	6.6	3.3	1.0	Tr.	0	Lig. of hepatic ducts to 2/3 liver. 24 hours

mals were still under anesthesia and before any bilirubinemia due to the obstruction was present. The 5 minute levels are about the same as in normal animals but there is no subsequent appreciable decrease during the 2 hour period of the test. Similar results were obtained in two animals in which both ducts were ligated 72 hours previously and in one in which the common duct alone was ligated 24 hours previously. These three animals had a definite bilirubinemia at the time of the test. In one animal

the hepatic ducts draining an estimated two-thirds of the liver were ligated and the test run 24 hours later. The removal in this animal was rapid. This experiment not only serves as a control with respect to the possible effect of the previous operative procedures, anesthesia, etc., but also shows the remarkable factor of safety of the liver with respect to the removal of bilirubin that has been shown by various other methods. These results show that complete obstruction of the biliary ducts is followed immediately by an impairment of bilirubin removal and indicate that the removal of intravenously injected bilirubin accompanies and is due to its excretion in the bile.

4. *Bilirubin removal in dogs after reticulo-endothelial blockade.* As mentioned previously the 5 minute plasma bilirubin concentrations usually found represent approximately 40 per cent of the total bilirubin injected. This is in contrast on the one hand to the results which are obtained with the group of dyes suitable for blood volume determinations in which case

TABLE 4

Bilirubin clearance in dogs after reticulo-endothelial blockade

EXPERIMENT NUMBER	INITIAL PLASMA BILIRUBIN	BILIRUBIN INJECTED	PLASMA BILIRUBIN IN MGM. PER 100 CC.				
			5 minutes	30 minutes	60 minutes	90 minutes	120 minutes
		mgm./kilo					
27	0	5	5.0	3.3	2.5	2.0	1.8
28	0	5	4.3	2.4	2.0	1.7	1.5
29	0	5	5.0	4.5	4.0	3.6	3.3
38	0	5	4.5	2.0	1.5	1.4	Tr.
39	0	2.5	2.8	2.0	1.4	Tr.	0

approximately 100 per cent of the injected dye is present in the plasma at the 5 minute interval (9, 10), and on the other hand to the results with substances like bromsulphalein in which case only approximately 5 to 10 per cent of the injected dye is present in the 5 minute plasma sample. Although Saiki (11) has shown by quantitative determinations of the bile pigments in the bile that ultimately nearly all of the injected bilirubin is excreted in the bile, it is difficult to account for the initial loss of 60 per cent during the first 5 minutes in this manner. The possibility of this fraction of the injected pigment being temporarily sequestered in the cells of the reticulo-endothelial system must be considered. The evidence previously referred to which indicates that a bilirubinemia produces a retention of bromsulphalein would support such a view. The observations of Kanner (12) that histological studies fail to demonstrate significant storage of bilirubin in the reticulo-endothelial cells except in cases of long standing obstructive jaundice do not exclude this possibility. Accordingly observations were made on 5 dogs after the attempted blockade of these cells

by India ink. The results are shown in table 4. In these animals the "blockade" was produced by the repeated daily intravenous injection of 20 cc. of 8 per cent India ink for 2 to 5 days. These animals showed definite to marked retention of bromsulphalein prior to the bilirubin injections. The plasma bilirubin levels showed relatively little change from the normal in the 5 minute values although the subsequent removal was appreciably delayed. It is clear that "blockade" does not produce quantitatively as much impairment of the removal of injected bilirubin as it does of bromsulphalein. This does not necessarily mean that the reticulo-endothelial system is not concerned in the initial rapid removal of bilirubin from the blood stream. Victor, Van Buren, and Smith (13)

TABLE 5

Bilirubin clearance in dogs under influence of decholin (decholin—2½ cc. intravenously every 30 minutes for 4 doses)

EXPERIMENT NUMBER	INITIAL PLASMA BILIRUBIN	BILIRUBIN INJECTED	PLASMA BILIRUBIN IN MGM. PER 100 CC.					CONDITION
			5 minutes	30 minutes	60 minutes	90 minutes	120 minutes	
		mgm./kilo						
12	0	5	4.3	2.3	0.6	0	0	Normal
13	0	5	4.0	1.4	0	0	0	Normal
36	0	5	4.6	2.5	2.1	2.3	1.9	Anesthesia
37	0	5	4.2	1.7	1.5	1.7	1.7	Anesthesia
11	0	10	10.0	4.7	3.5	3.5	4.2	Anesthesia
17	0	10	8.5	5.8	5.0	3.7	3.5	Anesthesia
28	0	5	4.3	2.4	2.0	1.7	1.5	R. E. Blockade
34	0	5	4.0	4.4	5.1	5.6	6.3	Common and cystic D. Lig. 1 hour
35	0	5	5.0	5.2	6.2	6.2	6.2	Common and cystic D. Lig. 1 hour

have shown that India ink injections suppress the excretion of brilliant vital red in the bile, but Saiki (14) has reported that such injections do not suppress the excretion of injected bilirubin in the bile. India ink injections accomplish only a partial blockade of the cells of the reticulo-endothelial system and what is more important affect principally the littoral cells. Bilirubin, being a freely diffusible substance might very well be taken up by cells to which India ink is not accessible. The "blockade" experiments do not, however, enable us to explain the initial rapid drop in bilirubin. The possibility that the hepatic parenchymal cells may be concerned cannot be disregarded.

5. *Bilirubin removal in dogs under the influence of decholin.* If the removal of bilirubin from the blood stream is primarily related to its

excretion in the bile it should be possible to produce a significant alteration in the removal of injected bilirubin by stimulating the bile flow with a cholagogue. For this purpose decholin (20 per cent sodium dehydrocholate—Riedel-de Haen) was injected intravenously during the course of the test—four doses of 2.5 cc. each at 30 minute intervals,—in a series of animals. The results are shown in table 5. No appreciable acceleration of the rate of removal was seen in normal dogs, in dogs after reticulo-endothelial blockade or in dogs with biliary obstruction. There was some apparent acceleration of the rate of removal in anesthetized animals. This was quite striking in one instance. In one animal (expt. 16–table 2) the test was run under anesthesia alone and the removal was very slow. Several days later the animal was again anesthetized and decholin administered during the test. The rate of removal was distinctly better than previously (expt. 17–table 5). The absence of effect in normal animals is probably to be explained by the fact that the normal rate of removal is so rapid that slight changes could not readily be detected by the methods used. Acceleration of removal was not of course to be expected in the obstructed animals. There was, however, somewhat more of an elevation of the plasma levels in the two hour samples than was seen in obstructed dogs without decholin. Marengo and Massimello (16) reported that the injection of decholin into clinical patients led to an elevation of the blood bilirubin values. They attributed this to a mobilization of bilirubin from the tissues. Whatever the explanation may be it appears to be unlikely that the increases seen can be explained by the obstruction alone and furthermore tends to substantiate the view referred to previously, namely, that that fraction of the injected bilirubin which is unaccounted for in the 5 minute plasma sample has not all been excreted by the liver but has been temporarily removed from the blood stream by some other mechanism.

SUMMARY

The removal of intravenously injected bilirubin from the blood stream in dogs, with and without anesthesia, after ligation of the biliary ducts, after reticulo-endothelial "blockade" with India ink, and during the administration of decholin are reported. Bilirubin is removed very rapidly from the blood of normal dogs. The rate of removal is impaired by anesthesia, and is immediately prevented by biliary obstruction. Both of these observations indicate that the removal from the blood stream is related to its excretion in the bile. An initial rapid removal of bilirubin, which is apparently not accounted for by its excretion in the bile is reported. "Blockade" experiments were unsuccessful in attributing this rapid removal to the cells of the reticulo-endothelial system but the possibility that some of the cells of this system may be concerned was not excluded. The administration of decholin had little effect upon the clearance of

bilirubin except in the anesthetized animals in which it tended to restore the clearance rate which had been retarded by the anesthesia.

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GASTRIC SECRETION IN RUMINANTS[1]

D. L. ESPE AND C. Y. CANNON

From Iowa State College, Ames, Iowa

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The stimuli to gastric secretion are commonly divided into a cephalic phase, a gastric phase and an intestinal phase (1). Pavlov was one of the first workers to make a detailed study of the cephalic phase or psychic secretion (2) as it is commonly called. According to Dukes (3) "the stimuli arise largely because of a psychic state developed as a result of the sensations of eating." The impulses are discharged from a gastric secretory center to the gastric glands via secretory fibers in the vagi. The latent period for this secretion is, in the dog, usually four or five minutes. Although this phase of gastric secretion is said to be absent in ruminants (3), no sustaining experimental evidence has been published according to the best information available to the authors.

EXPERIMENTS AND RESULTS. To get experimental evidence as to the effect of certain stimuli on gastric secretion, three calves were used, each with a Pavlov pouch which emptied through a modified Dragstedt cannula (4). Trials were not run consecutively, but intervening normal feedings were allowed to avoid "conditioning" the calf to the "teasing" methods.

Calves were fastened in a crate for observation during the trial in a small room as free from disturbing influences as possible. After placing a calf in the crate the rate of gastric secretion from the Pavlov pouch was recorded for one hour to ascertain the normal rate for that day. In the absence of a mechanical recording device for measuring the gastric secretion, one man counted aloud the number of drops of gastric juice which fell from the cannula while another person, holding a stop watch, recorded the total number of drops which escaped each minute.

As soon as the one-hour record of secretion had been made, a third attendant held a pail of milk in front of the calf for a five-minute "teasing period" after which the calf was allowed to drink the milk. Records were made of the gastric secretion throughout the "teasing" period and also for an additional half-hour, in order to compare the cephalic phase of gastric stimulation, if there is such, with the gastric phase and perhaps part

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of the intestinal phase. Calves were made hungry at the time of the trial by feeding them very sparingly the night before.

The results of 63 such trials with calf 47A11 are shown in figure 1. The data shown by this curve have been combined into five minute periods for the sake of simplicity.

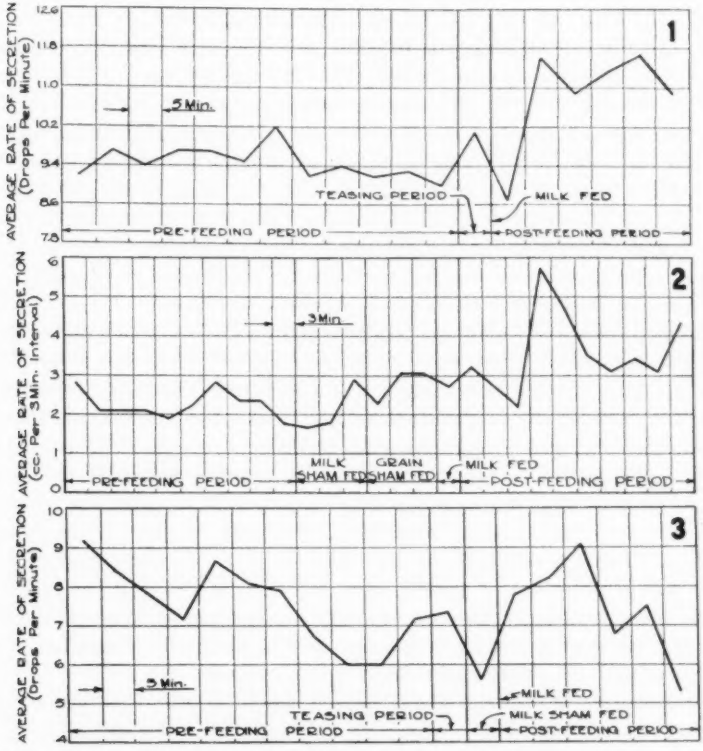

Fig. 1. Effect of different types of stimuli on rate of gastric secretion from a Pavlov pouch in a calf.

Fig. 2. Effect of different types of stimuli on rate of gastric secretion from a Pavlov pouch in a calf.

Fig. 3. Effect of different types of stimuli on rate of gastric secretion from a Pavlov pouch in a calf.

Modifications of the above procedure that were used with the second calf, no. 47A12, were as follows. An esophagotomy was performed when the calf was about four months old and the trials were begun when the calf was about six months old. Because the gastric juice did not drain from the Pavlov pouch in this calf at as consistent a rate as with the

previous calf, a rubber balloon was attached to the cannula which drained the pouch and the collections of gastric juice were recorded by three minute intervals. Another change in the procedure was the recording of gastric secretion for only one-half hour before the "teasing period" instead of one hour as was the outlined practice.

The method of eliciting a psychic response was also quite different from that used with calf 47A11. Instead of holding a bucket of milk in front of the calf, the calf was allowed to drink, but the milk was collected by an attendant from the esophageal fistula, in this way preventing it from passing to the stomach. This sham feeding of milk was continued during a nine-minute interval. Following the sham feeding with milk, another period of nine minutes was occupied with sham feeding of grain. An interval of three minutes elapsed following the sham feeding of grain to permit the insertion of a hollow rubber tube into the cut ends of the esophagus in order to make a conduit for normal feeding. At the end of three minutes the calf was fed normally its usual amount of milk. The results of ten trials with this calf are shown in figure 2.

A third calf, no. 47A36, was prepared with a Pavlov pouch and a fistula of the esophagus. These were healed and the calf ready to use when two months old. Since this calf's pouch secreted quite regularly, its secretory rate was recorded at one minute intervals. After the one-hour preliminary period this calf was teased with milk for five minutes and then sham fed milk a like period. The lower end of the esophagus was closed with a cotton plug so that it was impossible for any milk to enter the stomach. After five minutes of sham feeding the two ends of the esophagus were connected with a rubber tube and the calf given its usual feed of milk. The rate of secretion of gastric juice was recorded during the one hour preliminary period, the five-minute sham feeding period, through the time the calf was drinking its milk and for one-half hour after being fed. The results of five trials with this calf are shown in figure 3.

Discussion. From the above data it would appear that the psychic phase of gastric secretion is relatively unimportant in the calf although in some instances there was an increase of juice collected from the pouch during the teasing or sham feeding period. Some of this increase may have been due to a more complete emptying of the pouch when the calf was attempting to reach its food. This seems especially true in cases where there was a drop in rate of secretion immediately after teasing. Although the Pavlov pouch was supposedly open at all times the folds of the gastric mucosa doubtless tended to trap a slight amount of fluid. Any sharp movements of the calf or peristaltic movements of a viscus might temporarily influence the apparent rate of secretion.

Every effort was made in preparing the pouches to leave sufficient stomach wall intact at the juncture of a pouch and the balance of the abomasum

to prevent injury of the vagus fibers which are essential for the mediation of nervous impulses between the gastric center of the brain and the gastric glands of the stomach (5). In spite of this care, some injury to these nerve fibers might have occurred, though it seems improbable that the vagus was injured in every instance so that no nerve impulses could pass.

The reason for the rather minor fluctuations in rate of gastric secretion after feeding, as compared with animals like the dog, may be accounted for by the more continuous type of gastric secretion in ruminants (6). Normally, the rumen acts as a reservoir from which small amounts of finely macerated material are emptied into the abomasum at frequent intervals. Such a physiological mechanism would require a relatively constant flow of gastric secretion. Since little of the solid feed swallowed by the ruminant reaches the true stomach until some hours later the psychic phase of gastric secretion probably would not be essential.

SUMMARY AND CONCLUSION

Three calves with Pavlov pouches were used in the study of psychic secretion in ruminants. The results indicate that the psychic phase of gastric secretion in these animals is absent or of very minor importance. Either teasing with food or sham feeding where esophagotomies had been performed, were equally ineffective. Although calves normally show a marked preference for certain feeds, the sight or taste of these feeds should not appreciably accelerate the rate of gastric secretion over that obtained from less palatable feeds.

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THE NERVOUS CONTROL OF PANCREATIC SECRETION IN THE DOG

PHOEBE J. CRITTENDEN AND A. C. IVY

From the Departments of Physiology, Northwestern University, and of Pharmacology, George Washington University Medical School

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Although the secretion of pancreatic juice is undoubtedly influenced by extrinsic nerves (1–5), no attempt has been made to determine the secretory response of an animal to sham feeding in the absence of the secretin or hormone mechanism. Baxter (6) has shown that the "continuous secretion" of the pancreas continues from 4 to 5 hours after removal of the stomach and the small and large intestine in the decapitate and anesthetized rabbit. His experiments negated the assumption that the continuous secretion of the pancreas in the rabbit is due to a continuous humoral stimulus. We have extended his experiment to include observations on the "chronic" enterectomized dog. We have ascertained the response of the pancreas to sham feeding and the parenteral administration of glucose and saline solutions and certain drugs, and have attempted to ascertain if the nervous and secretin mechanisms for pancreatic secretion act synergistically.

METHODS. In order to remove the secretin mechanism dogs were anesthetized and the entire small intestine from the pyloric vestibule to the ileocecal valve was removed. A subserous resection of the duodenum was performed so as not to interfere with the blood supply of the pancreas. A large cannula was inserted into the stomach to permit drainage of gastric contents into a football bladder. The common bile duct was cannulated and the bile collected aseptically in a balloon. The accessory pancreatic duct was ligated and the main duct cannulated, the secretion being collected in a balloon. All the stomach was not removed because we (2) have been unable to demonstrate stimulation of the pancreas by the distention of, or the application of food or acid to the stomach, and to isolate secretin in significant quatities from the gastric mucosa (7) other than the antrum, and from the latter only in very small amounts. Mellanby and Hugget (12) obtained no secretin from the gastric mucosa. The previously evacuated colon was not removed because no food residues entered it after the operation. The animals received 500 cc. of normal saline containing 30 grams of glucose subcutaneously, twice daily at a definite time, at 8:30 or 9:00 a.m. and at 9:00 or 10:00 p.m.

The pancreatic secretion collected in the balloon was measured every half hour from 8:00 a.m. to 10:00 p.m. and then as a single collection from 10:00 p.m. to 8:00 a.m. In certain experiments the secretion was collected directly from the rubber drainage tube. The secretion was analyzed for total nitrogen and ash.

RESULTS. *Amount of secretion formed.* The continuous secretion collected under standard conditions, that is, six hours or more after the administration of parenteral fluids or sham feeding, was studied in eight animals. The amount varied from 0.2 to 9.0 cc. per hour in the different dogs.

The total daily output of secretion in the four animals which remained in excellent condition throughout the period of the experiment is shown in table 1. It varied from 14 to 147 cc. after the first postoperative day.

TABLE 1

Daily secretion of pancreatic juice by enterectomized dogs

DOG NUMBER	POST-OPERATIVE DAY	JUICE TOTAL	DOG NUMBER	JUICE TOTAL	DOG NUMBER	JUICE TOTAL	DOG NUMBER	JUICE TOTAL
		cc.		cc.		cc.		cc.
1	2	26.6	2	3.7	3	17.2	4	2.0
wt.	3	55.1	wt.	61.0	wt.	19.3	wt.	22.0
12	4	142.9	8.5	94.3	10	32.6	10.5	32.6
kgm.	5	147.9	Kg.	92.4	Kg.	37.1	Kg.	38.5
	6			56.0		36.9		38.0
	7			60.6		35.8		
	8					14.3		
	9					35.0		
	10					28.1		

Response to water and food. To determine the response to sham drinking and feeding the secretion was collected for several half-hour periods previous to exposure to the sham stimulus. Then water, milk and meat broth were sham fed.

Thirteen tests were performed with water on five of the animals. The secretion was augmented in six, not changed in one, and decreased in six tests. The increase in secretion was beyond the normal variation in the rate of the continuous control secretion in only one test. In this test the control average amounted to 1.5 cc. per half-hour; the secretion collected during the half-hour following sham drinking in the presence of thirst was 6.0 cc. After this period the secretory rate returned to a lower level. The results show that sham drinking is not a potent excitant of the pancreas.

Seven tests were performed with milk on five animals, the animals drink-

ing from 130 to 200 cc. An increase of secretion occurred in four and a decrease in two of the tests. The maximum increase amounted to a rise from 1.5 cc. during the control period, to 4.5 cc. during the one-half-hour sham feeding period. The results show that sham feeding of milk is not a potent excitant of the pancreas.

When meat broth was fed the rate of secretion was increased in eight of nine experiments conducted on three animals. Each of the three animals responded. The average control response prior to sham-feeding was 0.55 cc. per half-hour; during the half-hour sham-feeding period the average response was 1.55 cc.; during the following hour the average response was 1.1 cc. The type of response that occurred could only be interpreted as due to a psychic stimulation of the pancreas.

TABLE 2

Showing the total nitrogen and ash content of the secretion collected during the overnight and day periods

DOG NUMBER	TOTAL NITROGEN		ASH	
	Night	Day	Night	Day
	mgm. per cc.	*mgm. per cc.*	*mgm. per cc.*	*mgm. per cc.*
2	1.15	0.84	6.30	8.20
	1.39	1.57	4.25	3.65
	1.33	1.28	3.75	4.20
	1.00	2.29	1.15	2.75
3	3.25	3.03	2.10	1.15
	2.26	3.31	2.20	3.00
	3.25	3.03	3.00	1.75
	2.69	1.35	2.90	2.90
	2.08	2.36	2.60	2.20
4	4.05	2.65	2.25	2.00
	2.94	2.68	2.20	2.00
	3.15	2.78	2.10	1.40

Total nitrogen and ash content of the secretion. The secretion collected during the first 24 hours was not analyzed. After that period the secretions were analyzed before and after sham-feeding. The results were variable. In table 2 is shown the analysis of the secretion collected from 10 p.m. to 8 a.m. and a representative analysis of the secretion collected during the afternoon or sometime after the sham-feeding experiments. A significant trend is not evident.

The effect of glucose and normal saline solution. Because the animals received glucose and saline solution subcutaneously at definite intervals, we ascertained the effect of such solutions given intravenously on the secretion of the pancreas in these animals. Six per cent glucose (C.P.) in

freshly distilled water was given intravenously at the rate of 1.6 grams per kilo per hour. In two experiments (table 3, tests 3 and 5) the glucose was given for a period of 90 minutes. (The animals had been trained to lie quietly.) The results are shown in table 3. The variability in the results is evident; but it is clear that at first an inhibition of secretion usually occurs which is later followed by stimulation. The four- and fivefold increase in tests 3 and 5 cannot be attributed to spontaneous variation. The effect of normal saline intravenously is negligible. The results shown in table 3 are within the spontaneous variations observed during the course of this work with the exception of the inhibition to be noted in test 5 and in tests 2, 4 and 7 during the third half-hour period.

TABLE 3

The effect of glucose and normal saline solution given intravenously

DOG		GLUCOSE 6 PER CENT (1.6 GM./KG./HR.)				LATER EFFECTS 1 HR.	SALINE 0.9 PER CENT				AMOUNT GIVEN
		Control ½ hr.	During glucose				Control ½ hr.	During saline			
No.	Test		½ hr.	½ hr.	½ hr.			½ hr.	½ hr.	½ hr.	
		cc.	cc.	cc.	cc.		cc.	cc.	cc.	cc.	
2	1	2.0	2.0	0.2	1.6	2.5	1.5	2.0	2.5	0.9	6 cc./min.
	2	2.0	0.8	0.0	1.5	3.7	1.3	1.2	2.0	0.5	11.5 cc./min.
3	3*	0.5	1.7	0.7	2.5	3.3	0.5	1.6	1.3	1.0	12 cc./min.
	4	0.5	0.1	0.0	0.6	2.4	1.2	1.2	0.7	0.2	12 cc./min.
	5*	0.4	0.0	0.0	1.6	1.9	1.6	0.0	1.0	1.5	12 cc./min.
4	6	1.3	0.0	0.0	0.8	1.7	1.2	0.5	0.6	1.2	12 cc./min.
	7	0.9	0.0	0.2	0.8	2.0	1.0	1.0	1.0	0.3	12 cc./min.
	8	0.5	0.2	1.0	0.5	1.3	0.8	1.3	0.8	1.0	12 cc./min.

* Glucose solution given for 90 minutes otherwise the solutions were injected at the rate indicated for a period of one hour.

On analyzing the data on the rate of flow of the secretion as in the foregoing experiments, or by analyzing it during longer periods before and after the subcutaneous administration of fluid, it became evident that hydration or dehydration of the body was either adequately controlled or did not decidedly affect the quantity of the type of secretory response with which we were dealing. However, in all the dogs, following the subcutaneous injection given in the morning, a rise in the average hourly rate of the continuous secretion over that during the preceding night occurred in one-half the instances.

A study of the data on the total nitrogen and ash of the secretion collected before and after the administration of glucose and saline failed to yield a significant correlation.

Acetylcholine and eserine. Acetylcholine in doses of from 0.075 to 0.1 mgm. per kilo given slowly intravenously was injected five times in four of the animals. The secretion was increased only about twofold during the fifteen minute period following the injection in four of the five tests. Eserine in doses of from 0.06 to 0.1 mgm. per kilo was likewise injected in thirteen tests. Stimulation of the rate of secretion occurred eleven times. In five tests the augmentation was three or four times the control rate of secretion; 0.075 mgm. of eserine per kilo followed by the same dose of acetylcholine did not stimulate secretion. Smaller doses were not used.

In three experiments in two dogs acetylcholine increased the total nitrogen secreted. In four experiments on two dogs eserine increased the total nitrogen secreted; in one experiment no change resulted. The effect of these drugs on the secretion of ash was variable.

ACUTE EXPERIMENTS. Since the secretion formed by the pancreas of the animals in the foregoing experiments may be due in part to secretory effects exerted by the vagi, we deemed it advisable to repeat and extend certain experiments previously reported in the literature dealing with this subject.

In these experiments the animals were anesthetized with pentobarbital, the pylorus was obstructed and the pancreatic duct cannulated. In each instance the pancreatic response to a standard dose of vasodilatin free secretin was determined. The blood pressure was recorded in all the experiments, in which the vagus had been aspetically sectioned several days previously, to assure degeneration of the cardioinhibitory fibers.

Results. In the first place it was demonstrated that stimulation of the peripheral end of the freshly sectioned right or left vagus usually markedly depressed the flow of juice in response to secretin. This confirms Anrep (4), who demonstrated that the inhibition is due to the retention of secretion in the pancreas caused by contraction of the ducts.

We then determined the effect of stimulation of the vagus on the response to secretin in fourteen animals in which either the left or right vagus had been sectioned from 3 to 6 days previously. The plan of the experiment was as follows: The fasted animal was anesthetized, the pylorus obstructed and the pancreatic duct cannulated. The carotid artery was prepared for a blood pressure record. The peripheral end of the previously sectioned vagus was exposed for stimulation. The continuous secretion of the pancreas was recorded electrically. The response to a standard dose of secretin (0.5 mgm.) was determined. From 20 to 30 minutes later the peripheral end of the degenerating vagus was stimulated for five minutes. If stimulation of secretion was not obtained, the nerve was then stimulated again continuously or intermittently in the hope that a secretory response might be obtained. After we had ascertained whether the nerve would give an inhibitory or an excitatory effect only, we injected the standard

dose of secretin and at the same time started the electrical stimulation of the vagus. From 20 to 30 minutes later when the secretion had returned to the control rate, secretin was reinjected. The secretion of the same pancreas to a standard dose of secretin was quite uniform. The response of the duodenum to stimulation of the degenerating nerve was recorded, since we desired to ascertain if the motor fibers were still active.

The results are shown in table 4. The drops of secretion recorded are the drops in excess of the continuous secretion. In the fourteen experiments stimulation of the degenerated vagus resulted in stimulation in nine,

TABLE 4

Shows the result bearing on the question of the "synergistic action" of secretin and vagal secretory fibers

DOG NUMBER	VAGUS SECTIONED	DAYS AFTER SECTION	RESPONSE TO NERVE STIMULATION DROPS†	RESPONSE TO SECRETIN DROPS	RESPONSE TO SECRETIN PLUS NERVE STIMULATION DROPS	EFFECTS OF VAGAL STIMULATION PLUS SECRETIN STIMULATION
1*	Left	3	−3	10	26	Potentiation
2*	Left	4	+1	13	48	Potentiation
3*	Left	5	0	19	10	Inhibition
4*	Left	6	8	23	19	Inhibition
5*	Left	6	8	7	16	An additive effect
6*	Left	6	28	13	1	Inhibition
7*	Left	6	26	28	63	Potentiation
8	Left	6	5	49	4	Inhibition
9*	Right	5	0	6	3	Inhibition
10*	Right	6	8	13	9	Inhibition
11	Right	5	0	30	8	Inhibition
12*	Right	6	3	25	31	An additive effect
13*	Right	6	6	5	0	Inhibition
14*	Right	6	26	33	57	An additive effect

* Duodenal motility increased, showing the integrity of motor fibers to the duodenum.

† 15 drops yield 1 cc.

no change in four and inhibition in one (dog 1). In the three experiments (dogs 6, 7, 14) in which the greatest amount of secretion was obtained, the calculated secretory rate per hour amounted to from 5 to 6 cc. When the degenerating nerve was stimulated simultaneously with the action of secretin, we had anticipated a potentiating effect. A potentiating effect was obtained three times, an additive effect three times, and an inhibitory effect eight times. The inhibitory effect was unexpected.

In two instances the juice resulting from secretin, from nerve stimulation, and from nerve stimulation plus secretin was assayed for total nitrogen. The results were: (one test) secretin, 2.72 mgm. per cc.; nerve stimulation,

7.14; secretin plus nerve stimulation, 4.72; (the other test) secretin, 5.42; nerve stimulation, 9.9; secretin plus nerve stimulation, 6.41.

Response to acetylcholine and eserine. In the animals with one degenerating vagus nerve used in the foregoing experiments, the response of the pancreas to acetylcholine and eserine singly and in combination was studied. Acetylcholine in doses of 0.1 mgm. per kilo given slowly intravenously augmented the continuous secretion by five or more drops in 7 out of 38 tests on the 14 animals. Smaller doses were not effective. Inhibition of secretion occurred in five tests. Eserine in doses of from 0.06 to 0.2 mgm. per kilo stimulated the flow of secretion in 16 out of 38 tests, from 4 to 40 drops of secretion being obtained. No increase over the rate of continuous flow occurred in 9 tests. In the remainder, the secretion was only slightly augmented. Eserine apparently sensitized the pancreas to the secretory effect of stimulation of the degenerating vagus in only three out of nine tests; eserine sensitized the inhibitory action of the vagus in two of the nine tests. Acetylcholine given after eserine yielded variable results. In some instances (five) the eserine caused an augmentation of the secretory response to acetylcholine; in most instances, after eserine, acetylcholine caused the production of less secretion than it did previously. The nature and variability of these results indicate that both an excitatory and an inhibitory mechanism exists in the pancreas, that eserine is more selective for the excitatory mechanism, and that acetylcholine may excite either mechanism, but tends to select the inhibitory mechanism after an injection of eserine.

Atropine and pilocarpine were not used in these experiments because their effect on the pancreatic secretion in the dog is well known. Acetylcholine does not stimulate pancreatic secretion in the skate (13), but has a slight stimulating action in the rabbit (6).

Discussion. The results on the enterectomized animals demonstrate that the pancreas of the dog secretes an appreciable quantity of juice in the absence of the secretin mechanism and of the absorption of secretogogues elaborated by digestion. The failure of the glucose and saline solution to stimulate the secretion of the pancreas in the enterectomized animals to an appreciable extent shows that the secretion we collected was not principally due to the water, glucose, and salt administered for the purpose of sustenance. The results do not show whether the continuous secretion obtained was due to nervous stimulation of the cells of the gland or to the mere passage of blood through the gland, or both. To rule out extrinsic nervous influences the splanchnics, vagi, and the coeliac ganglion would have to be removed.

In those instances in which glucose caused a delayed slight increase in secretion, the increase was probably due to the posthyperglycemic stimulation that, according to La Barre (10), is due to a central nervous effect

transmitted to the pancreas by the vagi. This is best seen when the pancreas is secreting in response to secretin (11). Our results show that a secretin mechanism is not necessary for the response.

The results on sham-drinking and feeding demonstrate that the reflex psychic or cephalic stimulation of the pancreas occurs in the absence of the passage of gastric juice into the intestine, which confirms Tonkich (8). They also show that the psychic stimulation of the pancreas is not due to reflex stimulation of secretin production. This is in line with the results of Nakagawa, Kakita and Matsumato (9) who demonstrated that the secretion of the pancreas which follows electrical stimulation of the vagus nerve occurs in the absence of the intestine. The more uniform stimulation observed when meat broth was sham fed instead of milk and water indicates that the response, though small, was a true reflex, "psychic" response. In connection with the very small increase that occurred on sham-feeding, it must be remembered that even in a chronic pancreatic fistula dog, otherwise intact, the response to sham-feeding rarely amounts to more than an increment of 5 cc. in fifteen minutes.

The secretory response of the pancreas of the enterectomized animals to acetylcholine and eserine was relatively slight. Relatively large doses had to be employed to provoke stimulation, doses that produced defecation, salivation and in some instances vomiting. Since vomiting is known to depress pancreatic secretion (1), the results are difficult to interpret. Both drugs increased the total nitrogen in the secretion, an effect similar to that caused by pilocarpine.

The acute experiments were performed principally for two reasons: *first*, to ascertain whether the secretion collected from the chronic fistula animals could be due to a vagal secretory effect alone; and *second*, to ascertain whether the secretory activity of the vagus operated additively or in a potentiating manner with secretin. In the three dogs which responded best to stimulation of the peripheral end of the degenerating vagus, the secretory rate per hour amounted to from 5 to 6 cc. This amount is adequate to account for the maximum quantity of juice secreted in 24 hours by the chronic fistula dogs, and is obtained in response to the artificial stimulation of only one vagus. In addition, the sympathetic nerves under certain conditions exert a secretory effect on the pancreas (1). So, it is possible that the secretion collected from the chronic fistula dogs was formed solely under the influence of secretory nerve fibers in the vagi and splanchnics. In regard to the second question, our results show that the excitatory secretory fibers in the vagi for the pancreas may act either in an additive or a potentiating manner with secretin. However, even after six days of degeneration inhibitory fibers may still be present in the vagus and not manifest their presence until a second stimulation of the nerve is applied along with an injection of secretin. Is it possible that

secretin may in some way tend to lower the threshold of the inhibitory nervous mechanism, which Anrep (4) locates in the musculature of the ducts? Our observations on the effect of stimulation of the degenerating vagus on duodenal motility were made with this question in mind. The results observed did not answer the question.

SUMMARY AND CONCLUSIONS

1. The pancreas of the dog secretes continuously in the absence of the secretin mechanism and the absorption of secretagogue. The amount of juice secreted continuously under such conditions amounted to from 0.2 to 9 cc. per hour in eight animals. The amount secreted during twenty-four hours varies widely, a maximum of 147 cc. being obtained. Normal saline solution (12 cc. per min.) and 6 per cent glucose solution (at a rate of 1.6 grams glucose per kilo per hour) intravenously did not significantly alter the pancreatic response. This does not imply that in the presence of dehydration normal saline will not augment the rate of secretion. The mechanism responsible for the production of the continuous secretion was not determined. Yet, the data obtained by stimulating the degenerating vagus nerve, which contains secretory fibers, show that the secretion observed may be accounted for by the effect of excitatory secretory nerves on the pancreas.

2. A "psychic secretion" of pancreatic juice may be obtained in the absence of the secretin mechanism and the absence of the passage of gastric juice into the intestine. The quantity of juice secreted in response to sham-feeding is small, which is also known to be true when the ordinary pancreatic fistula dog is sham fed.

3. Eserine, like pilocarpine, usually stimulates the secretion of the pancreas slightly and causes the production of a juice rich in nitrogenous substances. Our results indicate that acetylcholine may effect either the excitatory or inhibitory mechanism of pancreatic secretion, but tends to select the inhibitory mechanism when given after eserine.

4. On stimulating the degenerating vagus nerve, at a time (3–6 days) when the inhibitory fibers to the heart have lost their irritability, an increase in pancreatic secretion is usually observed. Under this condition when the nerve is stimulated after the injection of secretin an additive or a potentiated secretory response may be observed. However, in most experiments (8 out of 14) inhibition of the response to secretin occurred, indicating that inhibitory fibers for the pancreas were still present in the degenerating nerve. The observation made by Anrep, that stimulation of the peripheral, freshly sectioned, end of the vagus inhibits the secretory response to secretin, was confirmed.

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THE INFLUENCE OF DEXTROSE ADMINISTRATION ON THE UTILIZATION OF β-HYDROXYBUTYRIC ACID BY THE NORMAL AND EVISCERATED RABBIT[1]

I. ARTHUR MIRSKY AND R. H. BROH-KAHN

From the Department of Metabolism and Endocrinology, Institute for Medical Research, Jewish Hospital, Cincinnati, Ohio

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Carbohydrate plays an important rôle in the metabolism of ketone bodies and their precursors, the fatty acids. This is indicated by the fact that the administration of carbohydrate results in the prevention or cessation of ketosis in human subjects (1) (2), in phlorhizinized dogs (3), and even in the fasted, completely depancreatized dog receiving no insulin (4) (5) (6).

It is obvious that the ketone body content of the blood and urine is dependent upon the difference between the rate of ketone formation and the rate of ketone utilization. Hence, any reduction in the ketosis consequent to carbohydrate administration must be due to either a decrease in the rate of the former ("antiketogenesis") or to an increase in the rate of the latter ("ketolysis"). From this point of view it is still an unsettled question as to whether carbohydrate is antiketogenic in the sense that it prevents the formation of ketone bodies, or whether it is ketolytic in that it accelerates the utilization of these substances.

The studies of Shaffer and his associates (7) (8), suggested to them that oxidizing glucose enters into some chemical reaction with acetoacetic acid and thus accelerates the oxidation of the ketones. As a result of their observations they postulated the existence of a definite "ketolytic" ratio between the hexose and fatty acids. However, there is some very good evidence that neither the administration of dextrose to the intact animal (9) (10) nor the administration of insulin to the hepatectomized animal (11) (12) has any appreciable effect on the utilization of injected acetoacetic acid. Furthermore, Chaikoff and Soskin (12) have demonstrated that there is no appreciable difference between the rates of ketone body utilization by the extrahepatic tissues of the normal animal and those of the depancreatized animal.

In order to obtain more information on this question, we set out to study the utilization of ketone bodies in a variety of conditions where

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the status of carbohydrate utilization is definitely established. This can be ascertained in the eviscerated rabbit by determining the rate at which glucose is removed from the blood stream (13). The rate of oxidation of injected ketone bodies can be studied in a similar manner (10) (11). Furthermore, the rôle of the liver and viscera in the utilization of ketone bodies can be inferred by comparing the results obtained in nephrectomized normal animals with those of completely eviscerated animals. If carbohydrate has a definite ketolytic effect, the administration of gucose should result in an acceleration of the removal of ketones from the blood. On the other hand, if carbohydrate administration is ineffective in this regard the action of glucose in a naturally occurring ketosis must be due to a cessation of ketone formation.

Because of its stability, ease of preparation, and the fact that about 75 per cent of the ketone bodies of the blood are present in the form of β-hydroxybutyric acid, a neutralized solution of 10 per cent i-sodium β-hydroxybutyrate was employed in these studies. Since the reaction acetoacetic acid $\rightleftarrows$ β-hydroxybutyric acid is a reversible one (14) the ketonemia produced by the administration of sodium β-hydroxybutyrate probably is very similar to that observed in other forms of ketosis.

Methods. Male white rabbits of approximately two kilograms body weight were employed in these studies after a preliminary fasting period of twenty-four hours. In all instances the femoral artery and vein were exposed under nembutal anesthesia. Two types of preparations were studied; *a*, nephrectomized rabbits, and *b*, completely eviscerated rabbits prepared as described in a previous paper (13). From 800 to 900 mgm. sodium β-hydroxybutyrate per kilogram of body weight were administered intravenously to both sets of animals. Fifteen minutes were allowed for diffusion; then arterial blood samples were drawn at half-hourly intervals and the total ketone content of the blood determined by the Van Slyke and Fitz method and blood glucose by the Somogyi modification of the Shaffer-Hartmann method.

One set of nephrectomized rabbits served as controls for the second group to which two grams of dextrose per kilogram of body weight were administered intravenously together with the β-hydroxybutyric acid. All eviscerated rabbits received an injection of 0.2 gram of dextrose per kilogram of body weight immediately after evisceration in order to maintain the blood sugar at a relatively high initial level. Since the rate of glucose removal from the blood of the eviscerated animal apparently is related directly to the initial level (i.e., the per cent drop from the initial level is fairly constant) (13) (15), it was not considered necessary further to increase the height of the blood sugar with injections of large amounts of glucose as was done in the nephrectomized rabbits.

Results. Our data are summarized in table 1 and reveal that glucose

administration has no significant effect on the removal of ketone bodies from the blood of the nephrectomized rabbit. Thus we found that the average loss of ketone bodies from the blood of the control group (A) was 43.5 mgm. per cent in one hour, while the glucose-treated group (B) lost 40.0 mgm. per cent during the same interval. These figures are

TABLE 1

β-hydroxybutyric acid utilization

A = Nephrectomized rabbits—without glucose administration
B = Nephrectomized rabbits—with glucose administration
C = Eviscerated rabbits.

ANIMAL NUMBER	BLOOD SUGAR			BLOOD "TOTAL ACETONE"*		
	Initial	30 Minutes	60 Minutes	Initial	30 Minutes	60 Minutes
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
A1	119	102	165	79.6	54.3	31.2
A2	96	178	320	83.7	55.6	38.8
A3	100	138	246	76.7	45.4	29.7
A4	65	90	110	71.8	46.2	32.9
A5	86	143	168	44.7	23.4	10.6
A6	107	159	188	59.4	33.1	11.9
Average	96	135	200	69.3	43.0	25.8
B1	290	258	176	55.7	37.2	17.8
B2	370	308	209	65.9	43.4	23.9
B3	425	406	304	63.0	40.7	25.9
B4	428	360	295	69.3	48.0	30.8
B5	369	203	185	67.8	44.9	23.5
B6	225	198	162	59.7	40.8	20.7
Average	351	289	222	63.8	42.5	23.8
C1	93	75	60	76.7	41.0	25.6
C2	123	105	83	70.3	44.0	24.2
C3	91	89	72	62.2	44.0	26.5
C4	126	108	76	66.5	38.7	16.8
C5	115	83	53	57.8	32.9	19.0
C6	151	120	100	71.9	45.1	24.9
Average	117	97	74	67.9	41.0	22.9

* Expressed as β-hydroxybutyric acid.

approximately the same as that of the completely eviscerated group (C) where the blood ketones decreased 45.0 mgm. per cent in one hour.

The blood sugar changes are of great interest. The administration of sodium β-hydroxybutyrate resulted in a progressive hyperglycemia of the control nephrectomized group (A). This is not obvious in the glucose-treated group (B) because of the high initial blood sugar level and where

an average decrease of 129 mgm. per cent is observed in one hour. In the eviscerated group (C) we note a progressive drop in blood sugar (43 mgm. per cent in one hour) in spite of a ketonemia similar to that observed in the first group. It is obvious from these data the hyperglycemia of group (A) must be due to an increased hepatic glycogenolysis and not to an impairment in carbohydrate utilization.

In view of the fact that the removal of blood ketones, like that of the blood sugar of the eviscerated animal, appears to be determined by the initial level, we computed the average rate of disappearance of both blood sugar and blood ketone bodies as per cent drop from the initial level (fig. 1). The average ketone body drop in one hour was 62.8 per cent for group A, 62.7 per cent for group B and 66.2 per cent for group C. By similar analysis we find that the blood sugar of the eviscerated group fell 36.7 per cent from the initial level in one hour. In order to evaluate the effect of the ketonemia on the rate of glucose removal we performed a series of experiments in which the rate of glucose removal from the blood of six untreated eviscerated rabbits was determined (fig. 1D). These

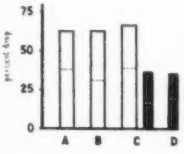

Fig. 1. Graphic comparison of the rates of disappearance of ketone bodies (white blocks) and of sugar (black blocks) from the blood of nephrectomized rabbits without glucose administration (A) and after glucose administration (B), and in eviscerated rabbits (C) (D). The dotted lines represent the half-hour interval, the top of block represents the one hour interval. Each block represents the average per cent drop from the initial blood sugar level.

revealed an average drop of 35.6 per cent from the initial blood sugar level in one hour. Comparison of these results reveals that the presence of ketone bodies in the blood does not impair glucose utilization.

In view of the fact that we employed a racemic mixture of β-hydroxybutyric acid, it is interesting to note that both the d- and l-forms are utilized as indicated by the removal of more than 50 per cent.

In summarizing the results, it is apparent that: 1, the ketonemia produced by the injection of sodium β-hydroxybutyrate is associated with a rise in the blood sugar of the normal animal but does not prevent a drop in the blood sugar of the glucose-treated animal; 2, the ketonemia produced in this manner does not affect the rate of sugar disappearance from the blood of the eviscerated animal; 3, the rate of β-hydroxybutyric acid removal from the blood is the same in normal, in glucose-treated and in completely eviscerated rabbits, although the milligram per cent loss of glucose from the blood varies markedly in the different preparations.

Discussion. Using the tissue-slice technique, Quastel and Wheatley (16) demonstrated that the acetoacetic acid was not further oxidized by the liver, just as Snapper and Grunbaum (17) observed with perfused

livers. Our observations and those of Chaikoff and Soskin (12) indicate that this is also true for the intact animal since the utilization of ketone bodies is no greater in the intact animal than in the case of the eviscerated preparation. Therefore, it is evident that the liver does not utilize ketone bodies. Moreover, it is obvious from our data that even the glycogen-rich liver (after glucose administration) is unable to oxidize ketones. Accordingly, it is apparent that the ketone bodies are normal end-products of fat metabolism in the liver.

The present study definitely indicates that the administration of glucose does not increase ketone utilization of either the liver or the extrahepatic tissues, i.e., it is not ketolytic in action. Therefore, the effects of glucose in inhibiting diabetic (5) or non-diabetic ketosis must be due to an actual inhibition of ketone formation, i.e., a true antiketogenic effect. In view of the probability that the ketone bodies are end-products of fat metabolism in the liver, the antiketogenic action of glucose must be due to a suppression of fat oxidation. In other words, *instead of facilitating fat oxidation, carbohydrate actually inhibits it.* These considerations are supported by the studies of Quastel and Wheatley (16), Walthard (18), Edson (19), and others. Quastel and Wheatley demonstrated that the addition of even-numbered fatty acids to liver slices gave rise to an increased oxygen consumption and acetoacetic acid formation. Neither the addition of glucose nor of lactic acid affected these results. However, in some instances, when glycogen was added to the medium, there was a decrease in the production of acetoacetic acid together with a decrease in oxygen consumption (i.e., fatty acid combustion). Walthard observed that glycogen-poor livers have a very high respiration whereas glycogen-rich livers have little. In accord with these observations are those of Edson who found that the glycogen-poor liver is more ketogenic than is the glycogen-rich liver.

Since fat oxidation in the liver probably never goes beyond the ketone body stage, the respiratory quotient for this process in the liver cannot attain a value as high as 0.7 but is more likely in the neighborhood of 0.3 (20). Hence, the value of 0.7 observed in pancreatic diabetes, starvation and other conditions is determined not solely by the fat oxidation. It is probable that utilization of carbohydrate by the muscles in these conditions (15) (21) is at least one other factor in determining this ratio.

CONCLUSIONS

The administration of carbohydrate does not influence the utilization of ketone bodies and therefore does not exert a ketolytic effect.

The presence of a ketonemia does not impair the utilization of carbohydrate.

The mechanism of antiketogenesis is discussed and it is postulated that the ketone bodies are end-products of fat metabolism in the liver.

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TIME RELATIONS IN RENAL EXCRETION OF THRESHOLD AND NO-THRESHOLD SUBSTANCES

H. L. WHITE AND THOMAS FINDLEY, JR.

From the Departments of Physiology and of Medicine, Washington University School of Medicine, Saint Louis

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Verney and collaborators (1) have postulated that the blood changes which follow the ingestion of water do not affect the kidneys directly but inhibit posterior pituitary secretion; the resultant hypopitressinemia is thus responsible for the diuresis, and the lag of urine output behind blood dilution or water load represents the time required for preformed pitressin to drop below its effective antidiuretic concentration. Various reports purporting to give direct evidence that the blood content of pitressin is diminished on taking water have appeared within the past few years but they have been denied by others; the present status of this question does not permit a decision. The present paper presents evidence that the hypopitressinemia theory of water diuresis appears unnecessary. It is shown that a similar lag exists in the output of two threshold substances other than water, namely, glucose and chloride, while there is no such lag with the no-threshold substance, creatinine. It therefore becomes probable that a lag in the increased output in response to increase in plasma level is characteristic of threshold substances in general and does not exist with no-threshold substances.

Methods. Normal human subjects were used in all experiments; the daily salt intake was not measured but was unrestricted. The studies were begun in the morning without breakfast and with previous water intake unrestricted. During a preliminary period urine collections were made at 15 or 20 minute intervals and one or more blood samples taken. The subject then drank the water or salt solution, and urine and blood collections were continued for several hours. The blood was collected without stasis from an arm vein into a dry syringe, transferred to a dry test tube containing a few glass beads and shaken to defibrinate and to equilibrate with room air.

Earlier experience with the gravimetric method for total solids and with the refractometer had indicated that these techniques were not adequate, in our hands as in others', to demonstrate reliably the small changes in serum protein concentration following water drinking. With the Bingham type of viscometer (2), employing appropriate facilities for maintaining constant temperature (25 ± .002°C.) and pressure head, we have been able to determine relative viscosities of serum with an error of less than ±0.10 per cent on samples of 0.6 cc. Calibration curves on

plasmas from three normal human subjects are shown in figure 1. The plasmas were diluted by 2, 5 and 10 per cent with 0.9 per cent NaCl solution and the relative viscosities plotted against protein contents, the original protein content having been determined by the Kjeldahl method. The protein-viscosity curves depart but slightly from a straight line within the range between undiluted and 90 per cent plasma and the slopes are such that a change of 0.1 in relative viscosity represents a change of 0.70, 0.72 and 0.74 gram per cent protein, respectively, or an average of 0.72. Since the figures for relative viscosity have an absolute error of not more than 0.002 we can detect changes of 0.014 gram per cent in protein content, or a change of 0.2 per cent. Errors inherent in the collection and sampling of the speci-

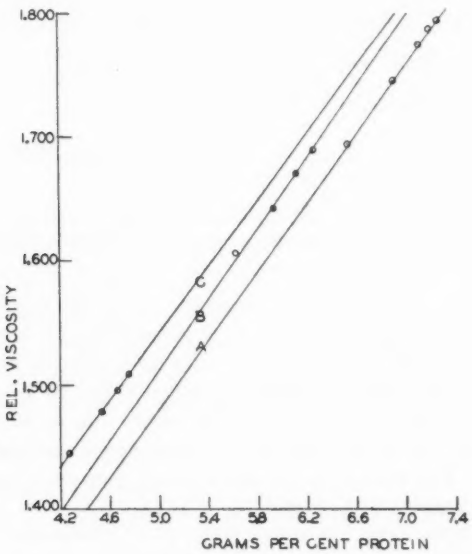

Fig. 1

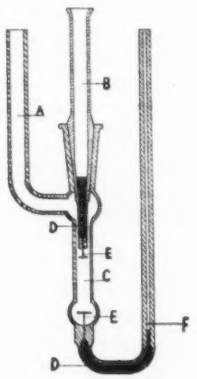

Fig. 2

Fig. 1. Viscosity-protein concentration curves on human plasmas.

Fig. 2. Conductivity cell. *A*, handle; *B*, male element of ground glass joint; *C*, fluid chamber; *D*, mercury; *E*, electrodes; *F*, lower electrode arm.

mens increase this slightly but we have shown that a change of 0.3 per cent in protein content, say from 7.30 to 7.28 grams per cent, is easily detected. The method is far more sensitive than is any chemical method applicable to small samples.

Conductivity determinations were carried out with a cell of the design shown in figure 2. The cell used requires about 0.4 cc. of material; its cell constant is 8.16, giving a resistance of 670 to 700 ohms with normal sera (3). Duplicate determinations always agreed to less than an ohm, or within 0.1 per cent; all were made at 25 ± 0.002°C. Since conductivity is influenced by changes in protein as well as in salt content, corrections have been made for the effect of changes in protein content where such changes have occurred as to entail a significant correction. Using our protein-viscosity factor we calculate the actual change in protein content; the effect

on conductivity is then calculated on Atchley and Nichols' (4) finding that each gram per cent of human serum protein in the neighborhood of pH 7.4 decreases conductivity by 1.58 per cent. The "corrected conductivity" shown in the figures thus means that which would obtain if there had been no change in protein content; i.e., it is an accurate representation of changes in salt content. This correction is never large on water drinking but is of some importance after salt solutions or with pituitrin.

A close approximation to the absolute magnitude of changes in plasma chloride concentration can be obtained from the conductivity figures. By graphic interpolation from the International Critical Tables we find that at 25°, $\Lambda = 105.9$ for 1.111 M or 0.65 per cent NaCl. By definition $\Lambda = 10^6\ K/C$ where K is specific conductivity in reciprocal ohms and C is concentration in millimols per liter. Then, for NaCl

$\Lambda = 5.846 \times 10^3\ K/C$, where C is concentration in grams per 100 cc.

$C = \frac{5.846 \times 10^3\ K}{105.9} = 55.2K$, where C is in grams per 100 cc.

$\frac{dC}{dK} = 55.2$, i.e., a change of 0.0001 in K means a change of 5.5 mgm. NaCl per 100 cc.

Urine chlorides were determined by a Volhard titration. Serum creatinine was determined by the Folin and Wu (5) procedure but using a ferric sulphate filtrate; urine creatinine according to Folin (6).

There are four series of experiments. The first three show the changes in serum viscosity (protein) and conductivity (chloride) and in urine water and chloride output *a*, after 1200 to 1400 cc. of water by mouth; *b*, after 1200 to 1300 cc. of 0.85 or 0.9 per cent NaCl; *c*, after 11 to 20 grams NaCl in 150 cc. water. In the fourth changes in serum concentration and urinary output of creatinine as affected by 4 to 10 grams of creatinine by mouth were followed. The data of a representative experiment from each series are shown in figures 3, 4, 5 and 6.

Results. 1. *Water.* After drinking water there is a definite fall in serum salt before water output increases (fig. 3). This fall begins promptly; in several experiments a blood sample taken 10 minutes after drinking 1200 cc. of water, where 5 minutes were required for the drinking, showed a fall in serum conductivity equivalent to 4 to 6 mgm. per cent NaCl and one blood at seven minutes a fall of 8 mgm. per cent NaCl, while there is rarely any increase in water output for at least 20 minutes. This electrolyte dilution has been a constant finding in 10 such experiments on 4 normal subjects; the greatest decrease has a peak at 50 minutes and has amounted to 17 mgm. per cent NaCl, while the maximum rate of water output occurred 100 minutes after the drink (averages of 10 experiments). The lag in increased water output behind the increased molar concentration of water in the serum, previously noted by other workers, is thus abundantly confirmed; in none of our ten experiments has it failed to be manifest.

The initial fall in protein content, as indicated by viscosity, is much

less than that of salt, but is beyond experimental error; samples taken between $3\frac{1}{2}$ and 5 hours after the drink show the protein well above the initial level (0.17 gram per cent average increase). An increase in rate of chloride output, seen in figure 3, is not a constant finding.

2. *Eighty-five hundredths per cent NaCl.* On taking 1200 to 1400 cc. of 0.85 or 0.9 per cent NaCl there is a prompt and large fall in plasma protein with a prompt and moderate rise in plasma salt (fig. 4). The rise in plasma salt may be seen within 15 minutes after ingestion while the increase in salt output never begins within 80 minutes; the crest of the plasma chloride increase precedes the crest of chloride output by 60 to 120 minutes (3 experiments on two normal subjects). The water content of the plasma is greatly increased, as shown by the fall of viscosity.[1] Even this degree of fall in plasma protein (as much as 0.3 gram per 100 cc.) does not, however,

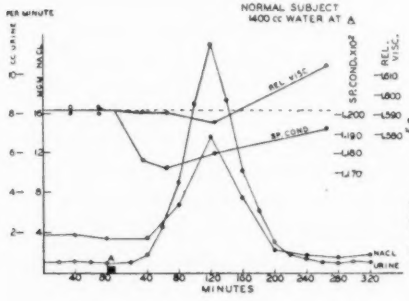

Fig. 3

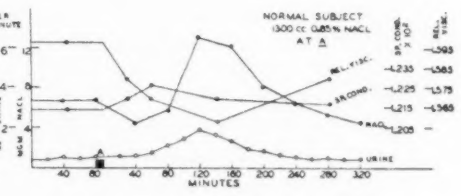

Fig. 4

Fig. 3. Response to ingestion of water.
Fig. 4. Response to ingestion of 0.85 per cent sodium chloride.

cause a dilution diuresis; the slight diuresis parallels and is presumably the result of the increased salt output.

The considerable and persistent rise in serum electrolytes and fall in protein has been a constant finding in our experiments on ingestion of 0.85 or 0.9 per cent sodium chloride. The increased output of water and chloride seen in figure 4 may, however, not appear until 3.5 hours after ingestion and may still be in evidence 6 hours after ingestion. As seen in

[1] The distinction between water content of plasma and mol fraction of water in plasma must be recognized. Thus, the percentage content of water in plasma (grams of water per 100 gm. of plasma) is increased by addition of a slightly hypertonic salt solution, while the mol fraction of water (fraction of total number of molecules and ions present represented by water molecules) is decreased. The latter determines the colligative properties of a solution; it is proportional to the aqueous vapor pressure and, under our experimental conditions, inversely proportional to the corrected specific conductivity.

figure 4 the kidneys need not (and usually do not) wait until the maximum concentration of urinary chloride has been achieved before responding to an increased salt output with an increased water output; water output may in fact increase (fig. 4) along with increased salt output so that urine chloride concentration is lower than initially, a labor-saving device on the part of the tubules.

3. *Hypertonic NaCl.* When a large dose of NaCl in a small volume of water is drunk there is a prompt and large fall in plasma protein comparable with that produced by 0.85 per cent NaCl, but the rise in plasma salt is much greater (fig. 5). These changes precede by 40 to 80 minutes any increase in chloride output. The lag in chloride output behind the increase in plasma chloride may be even more striking than shown in figure 5; i. e., there may be no increased output until the plasma chloride has reached

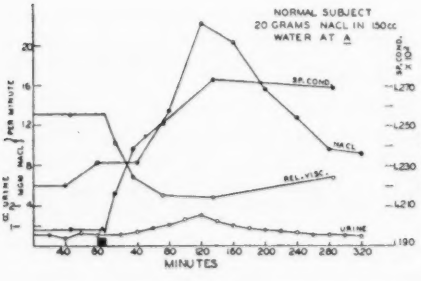

Fig. 5

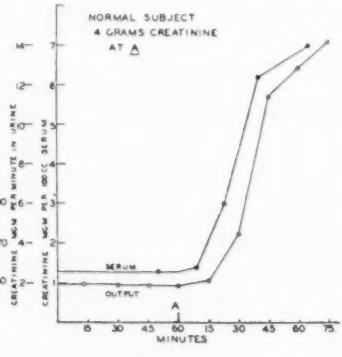

Fig. 6

Fig. 5. Response to ingestion of hypertonic sodium chloride.
Fig. 6. Serum creatinine and creatinine output.

its maximum. This has occurred when a smaller dose of salt (11 grams) was given; i. e., the maximum in plasma chloride was reached more quickly. After 20 grams (fig. 5) the rise in plasma chloride continues longer and has not yet attained its peak by the time chloride output has begun to increase but the plasma chloride rise always precedes the increase in chloride output by at least 40 minutes. Water output parallels chloride output, apparently a causal relationship. It is further noted that the tubules may become adapted to the higher plasma chloride since the chloride output returns toward the initial level while plasma chloride is still high; the same behavior toward water was noted in the water experiments.

4. *Creatinine.* In contrast to the lags in water and chloride output after increasing the plasma mol fractions of these constituents is the

prompt response of creatinine output to an increase in plasma creatinine (fig. 6). Here the first urine sample after ingestion of 4 grams of creatinine (taken at 15 minutes) shows an increased output and the output goes up along with plasma level.

DISCUSSION. Attention may be called to the fact first commented upon, so far as we know, by Faber and Hansen (7), that there is always a lag in the output of glucose behind the increase of blood sugar to the glycosuric level and that glycosuria may not appear until 30 to 45 minutes after the blood sugar has passed its crest. Since this finding appears so well established it has not seemed necessary to repeat the experiments.

It thus appears that tubular reabsorption will continue unabated for some time after a plasma level has been established which will eventually bring about a diminution of reabsorption; even normal tubules respond only sluggishly to changes in environment. Since the lag in output behind increased plasma concentration is seen to be just as pronounced for glucose and chloride as it is for water, it seems unnecessary to invoke the hypopitressinemia theory for the latter. This latent period apparently represents the time required for the tubules to adjust themselves to their new environment; it may be thought of as the time required for the renal parenchyma to achieve a new and higher equilibrium saturation with water, glucose, chloride or any other threshold substance, after which it rejects a higher proportion of these substances presented by the glomerular filtrate. In contrast, since tubular reabsorption of creatinine does not take place at any plasma level, an increase in plasma concentration is immediately reflected in an increase in rate of output.

Our findings, in addition to their rôle in establishing a difference in the time relations of tubular response toward changes in plasma concentrations of threshold and of no-threshold substances, are of interest from two other aspects; their bearing on theories of diuresis, and on changes in plasma volume following ingestion of water and of salt solutions.

They support the view of Priestley (8) (9), of Rioch (10), of Baldes and Smirk (11) and others that the diuresis of water ingestion is due primarily to an increase in the mol fraction of water in the plasma. The changes in plasma composition are so slight as to make it quite improbable that they would affect the behavior of the glomerular membrane. The initial fall in plasma protein is too small to change significantly the colloidal osmotic resistance to filtration. Although the point is still open to debate, it is not apparent how the changes in plasma composition after water ingestion could constitute an effective stimulus to glomerular vasomotor changes even though such procedures as splanchnic stimulation and adrenaline or caffeine administration may alter the glomerular filtering surface. It seems to us most probable that water diuresis is due primarily to a change in tubular rather than in glomerular behavior. Our conductivity findings

confirm the findings of others on serum conductivity, chloride concentration and vapor pressure that an acute fall in the total osmotic pressure of the plasma is the stimulus to a water diuresis; the tubules would be expected to be more sensitive than the glomeruli to such a change.

This view is supported by the finding that the clearances of creatinine and of inulin are independent of rate of urine flow. A point here requires comment. With a glomerular filtration rate of 100 cc. per minute and urine output of 0.5 cc. there are 99.5 cc. of water reabsorbed. If glomerular filtration rate increases to 110 cc. with 99.5 cc. still reabsorbed the urine output is 10.5 cc. per minute, a twenty-one-fold increase. The statement is sometimes made, on the above considerations, that even with what appears to be a relatively constant filtration rate a great diuresis may be of glomerular origin, the tubules being said to show a relatively constant behavior. But it cannot be too strongly emphasized that with the above data there is a great change in tubular function with no significant change in glomerular function in spite of the fact that the volume reabsorbed per minute has remained constant. The amount of work done by the tubules in reabsorbing water is much better indicated (inversely) by the percentage of filtrate escaping reabsorption than by that reabsorbed.

If one accepts the view that water diuresis is due primarily to a diminution in the water reabsorptive function of the tubules it is seen that, as Baldes and Smirk (11) have pointed out and as can be seen in the data of others, as Rioch (12), the tubules become adapted to the higher water mol fraction of the plasma so that water output falls to normal (tubular reabsorption of water rises to normal) while the mol fraction of water in the plasma is still above normal; an environmental change which had originally been an effective stimulus to diminish the water reabsorption function of the tubules ceases to be so. This has been a constant finding in ten such experiments; the mol fraction of water in the plasma, as indicated by conductivity, has never returned to normal as soon as the water output.

With glucose, on the other hand, according to Faber and Hansen and others, when tubular reabsorption has once become incomplete it remains so for some time after the blood sugar has fallen to an originally nonglycosuric level; the tubules show an inertia at both ends of the process.

The response of the tubules to ingestion of chloride is usually such that chloride output continues to rise for some time after plasma chloride has begun to fall; chloride output may remain above the initial rate for some time after the plasma level has returned essentially to its initial value, as is the case with glucose, or it may return to normal while the plasma level is still high, as is the case with water. In all experiments, however, a lag is seen at the beginning; i.e., chloride output never begins to increase until some 40 to 80 minutes after plasma chloride has begun to rise.

We are convinced that our viscosity data are more accurate than have

been obtained by other workers using the Hess or the Ostwald type of viscometer.[2] The only permissible conclusion of earlier workers on man has been that on physiological ingestion of water changes in serum protein, as indicated by refractometer, viscometer, total solids or Kjeldahl methods, were within the experimental error. We have been able to demonstrate a slight but consistent fall in viscosity (greatest at from 1 to 2 hours after ingestion and corresponding to 0.04 to 0.10 gram per cent protein) followed by a rise to above normal, the protein concentration at 3.5 to 5 hours after ingestion being about 0.17 gram per cent above the initial level. This late fall in plasma volume while the plasma is still diluted with respect to salt is probably due to the passage of water from plasma to cell, *cf.* Darrow and Yanett (18), and is consistent with Priestley's finding (9) that whole blood water content is increased by about 0.5 gram per cent on ingestion of 2000 cc. of water. The falls in serum protein with isotonic or hypertonic salt ingestion indicate corresponding rises in plasma volume.

SUMMARY

The Bingham type of viscometer is used on serum to detect changes in protein content as small as 0.02 gram per cent; calibrations on normal human plasmas show that a change of 0.01 in relative viscosity represents a change of 0.072 gram per cent in protein.

A small conductivity cell permits the detection of changes as small as 1 mgm. per cent of sodium chloride; a change of 0.0001 in specific conductivity of normal human serum represents a change of 5.5 mgm. per cent of sodium chloride.

[2] The only other plasma protein method of comparable accuracy is the specific gravity. Moore and Van Slyke (13) do not give data on duplicate determinations but it seems probable that determinations should show an absolute error of about ± 0.0002 in specific gravity; this would enable detection of a change of 0.07 gram per cent protein. The data of Bryan, Evans, Fulton and Stead (14) (upper half of their fig. 1) show much greater spontaneous fluctuations than this in normal control dogs but these are probably due largely to unavoidable stasis. We have found no such fluctuations in serum viscosity in controls on man, where blood is more easily collected but have found in controls on dogs fluctuations in serum viscosity indicating changes in protein concentration about half as great as those found by Bryan, Evans, Fulton and Stead. While the data of most workers fail to show any consistently measurable change in plasma protein in man on water by mouth, Bryan and collaborators find decreases as much as 1 gram per cent on 6.6 cc. water per kilo by mouth in dogs. This may be because the urine output of most dogs is less readily increased on water ingestion than is the case with man; failure to recognize this fact led Hashimoto (15) to conclude that distilled water by mouth was without diuretic effect on dogs. Smirk's (16) finding of a measurable fall in plasma protein on water by stomach tube in rabbits occurred with the large dose of 40 cc. per kilo.

The recent work of Evans and Gibson (17) strengthens the view that acute changes in plasma protein concentration as they occurred in our experiments reflect changes in plasma volume.

A lag in increased water output behind increased molar concentration of water in plasma is confirmed; a similar lag is found for chloride and attention is called to a similar older finding for glucose.

There is no lag in the increased creatinine output in response to an increased plasma creatinine.

The view is proposed that threshold substances in general show such a lag and that no-threshold substances do not. Since this lag is not peculiar to water it seems unnecessary to resort to the hypopitressinemia theory to explain the water lag.

An initial very slight fall in serum protein on ingestion of water is followed by a somewhat greater rise above the initial level. The rise occurs at a time when the serum is still diluted with respect to salt and is presumably due to passage of water from plasma to cells.

Water output always returns to normal sooner than does molar concentration of water in the plasma.

The earlier finding of a considerable and persistent rise in serum chloride and fall in protein on ingestion of isotonic or hypertonic sodium chloride solution is confirmed.

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PARADOXICAL EFFECT OF INTRAVENOUSLY INJECTED SODIUM CYANIDE IN SMALL DOSES ON HEART RATE AND BLOOD PRESSURE IN DOGS[1]

MILTON MENDLOWITZ[2] AND GERHARD SCHAUER

From the Laboratories of The Mount Sinai Hospital, New York, N. Y.

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During the course of experiments on the hemodynamics of coronary occlusion in the dog (1), the cyanide circulation time (2) was estimated by recording the time elapsing between the injection of 0.5 cc. of freshly-prepared one per cent sodium cyanide solution into the external jugular vein and the onset of deep rapid respirations. It was noted that, whereas the injection was followed in the normal animal by a transient bradycardia and hypertension, after denervation of the heart it was followed by a transient tachycardia and hypotension.

In order to analyze this effect, the reactions of the heart rate and the mean arterial blood pressure (mercury manometer) to the injection of this dose of sodium cyanide were noted in thirty-two dogs anesthetized with nembutal, under the following conditions: *1*, the normal dog; *2*, after bilateral sympatheticovagotomy[3] low in the neck; *3*, after bilateral sympathectomy (T 1–6)[4]; and *4*, after *2* and *3* combined, i.e., denervation of the heart, by which procedure the lungs and the great vessels in the mediastinum were also incidentally denervated. The effect of equivalent doses of sodium cyanide on the isolated canine heart perfused with oxygenated Tyrode solution was also recorded in several experiments.

RESULTS. In the normal dogs (fig. 1), the injection of sodium cyanide solution was usually followed by a rise in blood pressure. There was a tendency for the reaction to be biphasic, the initial rise being followed in some experiments by a slight, more prolonged fall. Occasionally, there was a preliminary short depression just before the initial rise. This was usually the result of an exaggeration of the respiratory waves by the suddenly increased respiratory amplitude, and in one instance it was due to temporary cardiac standstill. The predominant reaction, however, was the rise in pressure. This varied considerably in extent and in some

[1] Aided by grants from the Lucius N. Littauer and Walter W. Naumburg Funds

[2] Aided by a grant from the Emanuel Libman Fellowship Fund.

[3] In the dog, the vagosympathetic trunk includes the aortic nerve.

[4] Technique (1).

experiments was insignificant. On one occasion the only reaction was a fall in blood pressure. The low initial level here suggested a persistent depression of the carotid sinus and other vasosensitive areas by the anesthetic.

Of the three series of experiments in which preliminary nerve resections had been done, the greatest contrast to the normal was the group in which the heart had been completely denervated (fig. 1). Here, the predominant effect was a gradual fall in blood pressure occasionally preceded

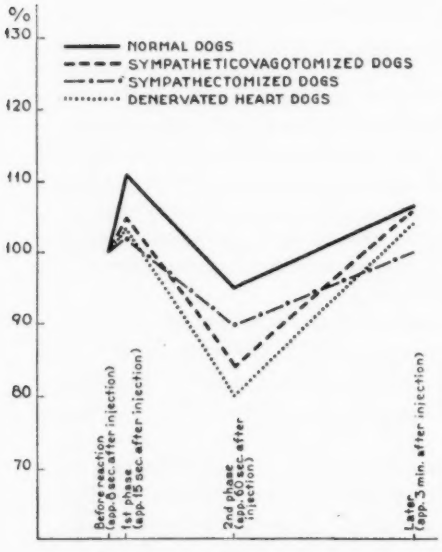

Fig. 1

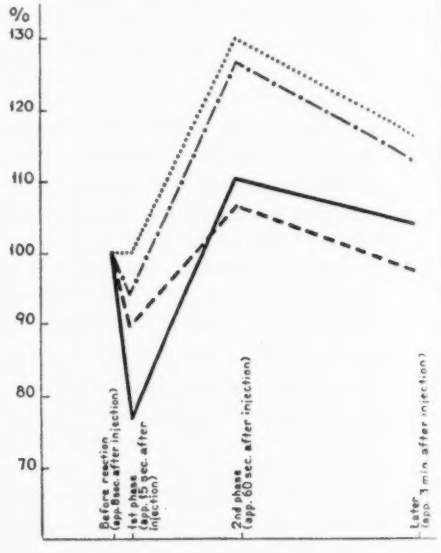

Fig. 2

Fig. 1. Average per cent changes in blood pressure after the injection of sodium cyanide.

Fig 2. Average per cent changes in heart rate after the injection of sodium cyanide.

by a slight rise. In both the sympatheticovagotomized (fig. 1) and the sympathectomized (T 1–6) (fig. 1) dogs, the effect of the injection on the blood pressure was more inconstant. The tendency for the reaction to be biphasic was more pronounced than in either the normal or the denervated heart groups.

The changes in heart rate were also biphasic. In the normal dogs (fig. 2) the injection was usually followed by a decrease in rate. This slowing, frequently coincidental with onset of the respiratory reaction, was occasionally followed by a moderate more prolonged acceleration. In

one experiment, the gradual acceleration was not preceded by any initial retardation.

In the three groups of experiments in which nerve resections had been done, the greatest contrast to the normal reaction was again found in the completely denervated heart series (fig. 2). Here, there was always a gradual quickening not masked by any initial slowing. The extent of the acceleration both here and in the sympathectomized (T 1–6) series might be accounted for, in part, by the relative bradycardia observed in both these groups before the injection. The reaction to the injection was variable in the sympatheticovagotomized (fig. 2) and sympathectomized (T 1–6) (fig. 2) groups. There was frequently an acceleration, partially masked in some experiments by an initial brief retardation.

In the experiments in which the isolated heart was perfused and made to beat regularly, the injection of 0.25 cc.[5] of 1 per cent sodium cyanide solution into the rubber tube through which the perfusate was forced into the coronary circulation was usually followed by an increase in rate and amplitude of contraction. When the ventricles were fibrillating, the injection was followed by a pronounced increase in the vigor of the fibrillations.

Discussion. With reference to blood pressure, the results represent an equilibrium between two opposing mechanisms, a reflex vasoconstriction and a direct vasodilatation. The former usually masks the latter in the normal dog. The direct effect may, however, assert itself as the reflex subsides, thus accounting for the biphasic reaction occasionally observed. If some of the reflex vasoconstrictor pathways are interrupted, as by bilateral sympatheticovagotomy alone or by bilateral sympathectomy (T 1–6) alone, such reflex vasoconstriction as occurs via the remaining pathways may in some instances be sufficient and in others insufficient to mask the more slowly developing direct vasodilatation. The resultant effect on blood pressure in these two groups is therefore quite variable. After both thoracic (1–6) sympathetic chains have been excised and both vagosympathetic trunks severed, there is never sufficient reflex vasoconstriction following the injection to mask the direct action of the drug to any great extent. The predominant effect is now, therefore, a hypotension only occasionally preceded by a slight hypertension.

With reference to heart rate, the results also represent an equilibrium between two opposing tendencies, namely, a reflex retardation and a direct acceleration. In the normal dog, the initial bradycardia predominates and frequently masks the direct tachycardia. The reflex slowing which occurs in the sympatheticovagotomized dog is the result of an inhibition

[5] This dose was selected because it elicited a physiological response without impairing conduction.

of sympathetic accelerator tone.[6] The reflex, however, lacks the vagal component and is therefore inadequate to mask the direct tachycardia. It occasionally appears as merely a delay in the onset of the acceleration. In the sympathectomized (T 1–6) animal, the inhibitory sympathetic component is no longer present. The reflex is again of insufficient intensity to mask the direct acceleration. The bradycardia can be entirely abolished only by complete denervation of the heart. The only reaction now is a direct acceleration.

It is possible that other factors are of importance in the initial phase of the reaction. The increase in respiratory rate is less marked and the increase in depth more marked in both groups in which the vagosympathetic trunks are severed. This factor may have modified the changes in blood pressure and heart rate to some extent. In the face of a bradycardia, it seems unlikely that adrenal secretion plays an important part in the initial hypertension, although this possibility cannot be entirely excluded in our experiments.

It is also necessary to consider the influence of other possible factors in the second phase of the reaction. The increase in heart rate observed after the injection in the sympatheticovagotomized and denervated heart groups is ample proof that we are not dealing with a Bainbridge reflex. The associated hypotension as well as the acceleration found in the isolated perfused heart experiments is evidence against the possibility that an increase in adrenal secretion plays an important part in the increase in heart rate. This possibility cannot here again be completely dismissed.

The consistent hypotension and cardiac acceleration in the denervated heart dog is persuasive evidence of a direct action of the drug on the heart and blood vessels. It is not necessary, however, to postulate a direct pharmacodynamic stimulation of the sino-auricular node or a direct pharmacodynamic dilatation of the blood vessels. These paradoxical reactions are attributable, we believe, to the transient depression of tissue oxidation by the cyanide radical (4). Such a depression is accompanied by an accumulation of metabolites as in exercise or in transient asphyxia. The vasodilatation is thus a consequence of this accumulation in the tissues in general (5), and the tachycardia a consequence of the same process in the myocardium, or more specifically, in the sino-auricular node.

Our findings in the normal dog are, with some exceptions,[7] in accord with those of other workers (2, 6, 7, 8, 9, 10). Pagano (11), whose work has been confirmed and extended by Heymans and his associates (12, 13), ascribes the rise in pressure and the bradycardia to a reflex vasoconstric-

[6] This is in accord with the view that the bradycardia of carotid sinus origin is due to an inhibition of sympathetic accelerator tone as well as an augmentation of vagal tone (3).

[7] Due to difference in dosage, species of experimental animal, or both.

tion and a reflex inhibition of the pace-maker respectively, initiated by stimulation of the carotid sinus and other vasosensitive areas by the drug. Although we have been unable to find any reference to the accelerating effect of the cyanide radical on the isolated, perfused canine heart, our results are in agreement with those found in other mammalian preparations (14). The paradoxical reactions in such experiments as well as in experiments in which a vasodilatation occurs in the isolated canine heart (15), liver (16), kidney (17) and leg (18), perfused with small doses of the drug, suggest that such reactions may be masked in the normal intact dog. Our results demonstrate that these paradoxical effects can be unmasked in the living animal.

SUMMARY

1. The intravenous injection of a small dose of sodium cyanide in an anesthetized normal dog is usually followed by a transient hypertension and bradycardia.

2. After denervation of the heart, the injection is usually followed by a transient hypotension and tachycardia.

3. The hypertension represents a reflex vasoconstriction and the bradycardia, a reflex augmentation of vagal tone and inhibition of sympathetic accelerator tone.

4. The hypotension represents a direct vasodilatation and the tachycardia, a direct cardiac stimulation.

5. In the normal dog, the reflex action of the drug on heart rate and blood pressure tends to mask the direct action.

6. The paradoxical direct effects are attributable to a transient depression of tissue oxidation by the cyanide radical.

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AN INTERMEDIARY CALCIUM COMPLEX IN BLOOD COAGULATION

JOHN H. FERGUSON

From the Department of Physiology and Pharmacology, School of Medicine, University of Alabama

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The present status of the calcium factor in blood coagulation was discussed in a recent review (3). Forty years ago Hammarsten (6, 7) oxalated and centrifuged thrombin and fibrinogen solutions until he obtained clots containing as little as 0.005 per cent of Ca. Although these "decalcified" reagents interacted slowly, the high potency of thrombin (Alex. Schmidt) is such that Hammarsten's experiments failed to rule out the possibility of calcium, bound to the protein, remaining as an integral part of the coagulant (8). Kastl's (10) preparation of an active thrombin by the alcohol precipitation of fresh (beef) serum after filtering off the calcium oxalate formed in 24 to 48 hours by the Kramer-Tisdall-Schimmelpfeng technique, has been supplemented by calcium analyses (13) from which it was claimed that calcium-free thrombin could clot calcium-free fibrinogen. Eagle (2) was also unable to inactivate thrombin by oxalation.

Opposing data include the following: Rabinovich (11, 12) claimed that electrodialyzed thrombin merely flocculated fibrinogen and required the addition of calcium salt for true clotting. Loucks and Scott (9) confirmed by Ferguson (5) showed that it was possible to inactivate thrombin (of plasma and tissue origin) by incubating with decalcifying anticoagulants (oxalate, etc.).

The following experiments were devised in order to elucidate the discrepancies in the cited literature.

EXPERIMENTAL TECHNIQUE. Details of the methods employed have been given in a previous communication (4). It may be noted that the methods include accurate measurement of the quantities of reagents used and the fullest possible controls, including thermostat water-bath (38°C.) for all incubations. The reagents used, in addition to standardized solutions of electrolytes ($CaCl_2$, sod. citrate, pot. oxalate, etc.), were *a*, Howell prothrombin from Berkefeld-filtered dog plasma; *b*, prothrombin-free (dog) fibrinogen, and *c*, an extremely potent pure brain cephalin. Clotting times under standard conditions (4) were taken as a measure of thrombic activity. The tests selected for the tables are representative of a large number of experiments.

RESULTS. Thrombin was prepared by mixing prothrombin extract, cephalin, and calcium chloride. Preliminary tests established the following empirical mixture as uniformly yielding a potent thrombin within a few minutes: *a*, prothrombin solution, 100 cc.; *b*, 0.1 per cent cephalin suspension, 10 cc.; *c*, N/10 $CaCl_2$, 10 cc. The calcium content could be lessened slightly without reduction of clotting power. The proportion of calcium noted, however, is in the neighborhood of the least amount required to give a maximally active thrombin in the time (table 1) usually allotted. The question of *minima*, *optima* and *maxima* both for calcium and for the

TABLE 1

Action of oxalate and citrate in the early stages of thrombin formation. Clotting-times for prothrombin-free fibrinogen. Dog. 38°C.

	TIME AFTER MIXING PROTHR. + CEPH. + CA	DILUTED THROMBIN (OXALATE CONTROL)	OXALATED THROMBIN (OXAL.:CA::120:1)	CITRATED THROMBIN (CIT.:CA::12:1)	DILUTED THROMBIN† (CITRATE CONTROL)
1	15 seconds*	1020 seconds	No clot	No clot	2010 seconds
2	30 seconds*	960 seconds	No clot	No clot	1800 seconds
3	45 seconds*	810 seconds	No clot	No clot	1290 seconds
4	1 minute*	690 seconds	No clot	Neglig. trace (3 hours)	840 seconds
5	2 minutes			1290 seconds	525 seconds
6	5 minutes	150 seconds	790 seconds	420 seconds	150 seconds
7	10 minutes	60 seconds	210 seconds	210 seconds	70 seconds
8	15 minutes	45 seconds	215 seconds	105 seconds	55 seconds
9	20 minutes	40 seconds	200 seconds	90 seconds	50 seconds
10	25 minutes	40 seconds	180 seconds		
11	30 minutes	35 seconds	180 seconds	95 seconds	55 seconds

* "Incubation" or "induction" period.

† The same prothrombin solution was used in all tests, but was 6 hours older in the citrate series, whence the slight difference between the two controls. Note the essentially similar effects of oxalate and citrate although the oxalate:Ca ratio was ten times the citrate:Ca ratio.

decalcifying anticoagulants has been investigated by previous workers (cited 3).

Table 1 demonstrates *a*, the rate of thrombin formation from control mixtures of prothrombin + cephalin + calcium salts, and *b*, the effects of excess of oxalate or citrate (added to the fibrinogen) during the early stages of prothrombin activation. It is observed from the controls that the maximal activity (represented by a clotting time of some 40–50 seconds in this particular experiment) was reached in 10 to 15 minutes. In other experiments a higher potency (shorter clotting time) was attained more quickly (perhaps 3 min.), the average time for attaining the maximum being 6 to 7 minutes, which is in substantial agreement with Cekada (1). The

maximal activity persisted at substantially the same level for many hours (even days in some experiments).

In the first minute (under the conditions of our experimentation) the coagulant mixture failed to yield a thrombin capable of clotting citrated or oxalated fibrinogen. This phase or zone may be termed the incubation ("induction," *Mills*) period. In the subsequent phases (generally coincident with maximal activation) clotting occurred in the presence of the decalcifying anticoagulants, albeit less quickly than in the controls which were simply diluted with corresponding amounts of water or 0.9 per cent NaCl solution. This retardation phenomenon may be termed the "immediate effect" of oxalation (or citration). The action of increasing amount of oxalate is illustrated in the data summarized in table 2; the correspond-

TABLE 2

The effect of increasing amounts of potassium oxalate upon the clotting-time of thrombin-fibrinogen mixtures. Dog. 38°C.

										AGE OF THROMBIN (AT START)
A	Ratio oxal.:Ca	0	4	8	16	32	40			17 min.
	Clotting-time	45″	45″	90″	150″	305″	325″			
B	Ratio oxal.:Ca	0	5.6	11.2	14	28	56	112	140	20 min.
	Clotting-time	20″	30″	35″	40″	95″	160″	215″	240″	
C	Ratio oxal.:Ca	0	7	14	28	35	70	140	280	10 min.
	Clotting-time	20″	20″	20″	25″	60″	105″	120″	165″	
D	Ratio oxal.:Ca	0	14	28	56	112	140			20 min.
	Clotting-time	35″	120″	165″	165″	140″	140″			

ing data for citrate, in table 3. The relative amounts of anticoagulants per mole of added calcium were computed stoichiometrically. Note that there is a progressive retardation of clotting as the oxalate or citrate increases. There is, however, a *maximum* beyond which some return to a shorter clotting time as usual, especially with citrate. The possibility that this is related to the clinically used hemostatic action (*v.* 3) of citrate injections (in dangerous dosage) is mooted.

Tables 4 and 5 show the effects of incubating variously treated thrombins with oxalate or citrate. The same test fibrinogen and the same prothrombin solution were used for all the tests of table 4, and for the series A, B, C of table 5. "Fresh" thrombin (i.e., about 10 min. from time of mixing) could be inactivated *progressively* by incubation with 50–100-fold excess of oxalate (4, B) or citrate (5, B). In several confirmatory experiments (e.g., 5, B′) complete inactivation was secured. "Ripe" thrombin

(i.e., several hours old), on the contrary, showed no impairment of clotting power except for the "immediate effect" —on incubation with oxalate (4, C) or citrate (5, C). By dialyzing "fresh" thrombin which had been

TABLE 3

The effect of increasing amounts of sodium citrate upon the clotting-time of thrombin-fibrinogen mixtures. Dog. 38°C.

											AGE OF THROMBIN (AT START)
A	Ratio cit.:Ca	0	1.4	2.8	5.6	7	14	28	56	70	15 min.
	Clotting-time	30″	30″	30″	35″	50″	150″	510″	360″	360″	
B	Ratio cit.:Ca	0	7	14	28	42	56	84			15 min.
	Clotting-time	15″	40″	80″	115″	130″	135″	100″			
C	Ratio cit.:Ca	0	14	28	56	112	140				30 min.
	Clotting-time	35″	90″	120″	120″	80″	100″				
D	Ratio cit.:Ca	0	16	32	64	80	96	128	160		12 min.
	Clotting-time	20″	35″	75″	120″	165″	150″	120″	100″		

TABLE 4

Effects of incubation with oxalate ($N/1\ K_2C_2O_4$) upon the clotting-times, for prothrombin-free fibrinogen, of various thrombins. Dog. 38°C.*

	PERIOD OF INCUBATION	DILUTED "FRESH" (12 MINUTES) THROMBIN	OXALATED "FRESH" (12 MINUTES) THROMBIN	OXALATED "RIPE" (4 HOURS) THROMBIN	OXALATED ELECTRO-DIALYZED THROMBIN	DILUTED ELECTRO-DIALYZED THROMBIN
1	0 (control)	15 seconds	15 seconds	20 seconds	75 seconds	
2	¼ minute	15 seconds	55 seconds	40 seconds	255 seconds	70 seconds
3	1 minute	20 seconds	60 seconds	50 seconds	240 seconds	
4	5 minutes	15 seconds	95 seconds	40 seconds	240 seconds	70 seconds
5	½ hour	15 seconds	160 seconds	45 seconds	240 seconds	90 seconds
6	½ hour	15 seconds	390 seconds	40 seconds	245 seconds	70 seconds
7	¾ hour	15 seconds	840 seconds	40 seconds	250 seconds	
8	1 hour	15 seconds	1380 seconds	60 seconds	265 seconds	100 seconds
9	1½ hours	15 seconds	2700 seconds	50 seconds	285 seconds	
10	2 hours	20 seconds	4500 seconds	50 seconds	250 seconds	70 seconds
		A	B	C	D	E

* Routine thrombin mixture (see text) + equal volume of N/1 oxalate. Electrodialysis 8 hours.

progressively inactivated by citrate to a clotting time of about 1 hour, we have been able to check the inactivation, and by recalcifying to restore the clotting-time to a value almost identical with a control consisting of untreated prothrombin with a like amount of calcium and cephalin.

Electrodialysis experiments. In three experiments "ripe" thrombin was electrodialyzed for 2 hours, 8 hours and 13 hours, respectively, in a Bradfield 3-compartment electro-dialyzer with collodion membranes. The 110-volt direct current reached a maximum amperage of 0.3 to 0.4 amps. in ¼ hour and fell to one or two milli-amps. in about 2 hours. Distilled water was run continuously through both electrode (end-) chambers. The prothrombin began to flocculate out at the *anode* membrane in about 15 minutes and sedimented to the bottom of the middle chamber. Later it was observed to undergo a partial cataphoretic migration to the *kathode* membrane. It is reasonable to suppose that the diphasic behavior was the result of the pH of the medium passing from the alkaline to the acid

TABLE 5

Effects of incubation with citrate (N/1 $Na_3C_6H_5O_7$) upon the clotting-times, for prothrombin-free fibrinogen, of various thrombins. Dog. 38°C.*

	PERIOD OF INCUBATION	DILUTED "FRESH" (10 MINUTES) THROMBIN	CITRATED "FRESH" (10 MINUTES) THROMBIN		CITRATED "RIPE" (3½ HOURS) THROMBIN		CITRATED ELECTRO-DIALYZED THROMBIN	DILUTED ELECTRO-DIALYZED THROMBIN
1	0 (control)	10 sec.	10 sec.	20 sec.	20 sec.	20 sec.	1½ min.	
2	¼ min.	15 sec.	110 sec.	105 sec.	315 sec.	215 sec.	10¾ min.	1¼ min.
3	1 min.	10 sec.	90 sec.	150 sec.	285 sec.	165 sec.	12 min.	
4	5 min.	10 sec.	135 sec.	195 sec.	285 sec.	150 sec.	11 min.	1¼ min.
5	½ hr.	15 sec.	195 sec.	210 sec.	310 sec.	140 sec.	11 min.	1½ min.
6	½ hr.	15 sec.	310 sec.	270 sec.	420 sec.	145 sec.	10¾ min.	1¼ min.
7	¾ hr.	15 sec.	510 sec.	330 sec.	465 sec.	90 sec.		
8	1 hr.	15 sec.	900 sec.	765 sec.	510 sec.	145 sec.	10½ min.	1¼ min.
9	1½ hr.	15 sec.	1290 sec.	840 sec.	610 sec.		9¼ min.	1½ min.
10	2 hr.	20 sec.	1800 sec.	No clot	625 sec.	145 sec.	10 min.	2 min.
		A	B	B′	C	C′	D	E

* Routine thrombin mixture (see text) + equal volume of N/1 citrate. Electrodialysis 13 hours.

side of the isoelectric point (?pH = 5.3) of the prothrombin. At the end of the dialysis neither the prothrombin nor the dialyzing fluids showed any trace of opacity with oxalate test solutions. Spectroscopic tests, kindly performed by Prof. B. A. Wooten and Mr. F. T. Allen of the Physics Department, revealed no trace of calcium in any of the electrodialyzed thrombins, nor in the test fibrinogen.

Added to the test fibrinogen, electrodialyzed thrombin (coarse suspension) caused gross flocculation (*c.f.* 11). On testing the pH with methyl red, it was found that the thrombin had become markedly acid. This could explain the flocculation, since the *isoelectric point* of fibrinogen, in some unreported tests of our own, confirmatory of other workers (cited 3),

was in the neighborhood of pH = 5.8. In some experiments the flocculation passed over into a clot, at first flocculant, but later solid. On "neutralizing" with dilute KOH (of the highest purity) to pH = 7.5 (phenol red), the precipitated thrombin completely redissolved and now gave excellent solid clots, uniformly. The water-clear supernatant fluid was always found to possess some (relatively weak) thrombic activity, although the precipitated material was much more potent. "Fibrinolysis" was noted several hours after clotting, as in Cekada's (simple) dialysis experiments on thrombin (1). Beyond suggesting that this could not be explained on the basis of bacterial action, we have no explanation to offer at this time concerning fibrinolysis. It is worthy of mention that progressively diminishing amounts of dilute KOH were required to neutralize the thrombin as the electrodialysis proceeded.

Incubation of electrodialyzed, "neutralized" thrombin with excess of oxalate (4, D) or citrate (5, D) gave no evidence of "progressive inactivation." In one exacting series of tests on the 13-hour electrodialyzed thrombin, both the thrombin and the fibrinogen were incubated for 18 hours with an equal volume of N/1 oxalate (and citrate) and good clots were obtained in about ½ hour in the final testing.

Addition of calcium salts, however, always improved the clotting efficiency of electrodialyzed thrombin, yielding firmer clots in a slightly shorter time.

Stability. The properties of plasma prothrombin were noted in a previous paper (4). In solution it was able to yield serviceable thrombin for several days, but it gradually deteriorated. Thrombin preparations, on the other hand, proved to be remarkably stable, giving good clots even after a couple of weeks in the ice-box. During the first few days after preparing the prothrombin-cephalin-calcium mixture there was hardly any loss of potency. On a number of occasions the thrombin preparation was *boiled* for 1 to 2 minutes and found to retain a considerable degree of activity. The electrodialyzed thrombin also kept well (for a week) and it, too, was not completely inactivated by brief boiling.

Conclusions. The present data, limited to plasma thrombin, reconcile the conflicting views in the literature on the rôle of calcium in blood clotting. It is clearly indicated that calcium forms an *intermediary complex* (prothrombin-cephalin-calcium "compound"). This complex soon passes over into a stable thrombin. The intermediary can readily be deprived of its calcium with resulting inactivation, whereas the final coagulant can be prepared calcium-free without significant loss in potency, provided that the pH is controlled when testing it with prothrombin-free fibrinogen. Our purified fibrinogen could not be electrodialyzed without complete denaturation (loss of coagulative function) and we have relied upon

thorough oxalation (and citration) to control the remote possibility that any small content of organic calcium (ref. 3) might influence the tests performed. It is obvious from her protocols (10) that Rabinovich's dialyzed fibrinogens were badly denatured.

The mode of action of the so-called "decalcifying" anticoagulants is now elucidated. In addition to their classical effect of *preventing* clotting by depression of the ionization of the calcium salts necessary for thrombin formation, they can progressively remove calcium from the "intermediary complex." This is strictly analogous to the yielding of calcium oxalate in the modern techniques for the determination of the calcium content (including "combined" calcium) of the protein-containing body fluids. Once the thrombin is "ripe" or fully elaborated, however, calcium is now no longer an essential component and oxalation or citration ceases to affect its potency, except for the usual non-specific "immediate effect." The nature of this immediate effect is not obvious and the possibility of certain minor actions, unrelated to the calcium factor, must remain an open question. It is not clear, also, exactly why calcium facilitates the action of electrodialyzed thrombin, unless it be that some unconverted prothrombin persists in the mixture.

SUMMARY

In a series of controlled experiments, plasma *thrombin* (prothrombin-cephalin-calcium mixture) *a*, "fresh" (few minutes); *b*, "ripe" (several hours), and *c*, electrodialyzed (calcium-free), was subjected to oxalation and citration before testing clotting activity on prothrombin-free fibrinogen. The decalcifying anticoagulants show three phenomena, viz.: 1, prevention of thrombin formation by depressing ionization of the calcium salts essential for the process of activation; 2, progressive inactivation of "fresh" thrombin, and 3, a characteristic immediate effect (varying with concentration) which is the sole action seen in tests on "ripe" and electrodialyzed thrombin. The evidence adduced points to a calcium-containing *intermediary complex* in the formation of thrombin. The activity of the "ripe" coagulant, however, is essentially independent of calcium.

It is a pleasure to acknowledge the assistance of Prof. B. A. Wooten and Mr. F. T. Allen of the Physics Department in the spectroscopic analyses for calcium, and the advice of Dr. J. L. Kassner of the School of Chemistry on the technique for electrodialysis. The experimental work was greatly aided by the technical assistance of Mr. J. M. Flowers.

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THE DIFFERENCE IN POTENTIAL ACROSS GASTRIC MEMBRANES AND CERTAIN FACTORS MODIFYING THE POTENTIAL

J. P. QUIGLEY, J. BARCROFT, G. S. ADAIR AND E. N. GOODMAN

From the Department of Physiology, Western Reserve University, Medical School, Cleveland, O. and the Physiology Laboratory, Cambridge University, Cambridge, England

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Sarre (1934) recorded difference in potential across gastric membranes of anesthetized cats when electrodes were applied to the mucosal and the serosal surfaces of the stomach. He demonstrated that as the acidity of the gastric contents increased, the potential of the serosal electrode became more positive. Such a result can be explained by assuming that Nernst's theory of diffusion potential applies to this system, since the positively charged hydrogen ions tend to move more rapidly than the negative ions from a fluid where their concentration is high to a fluid where the concentration is low.

We have repeated certain phases of Sarre's work with a modified method, which appears to avoid a number of objectional experimental factors, chiefly by permitting the use of unanesthetized animals. Under these conditions the application of acid to the mucosal surface did not produce the changes in potential described by Sarre. We investigated this subject in some detail and also tried various other procedures which might alter the potentials. The difference between our results and those of Sarre may be explained by the employment of different experimental methods.

METHODS. Employing two unanesthetized dogs provided with Pavlov pouches and trained to lie at ease on comfortable mattresses, the method of Adair and Goodman (1935) has been employed in 64 experiments to measure the electrical potential of the stomach and of the pouch. A combined stomach tube and electrode was introduced *via* the esophagus into the stomach and a second tube and electrode was placed in the pouch. The tubes were used in administering the test solutions and each extended 1 cm. beyond the end of the corresponding electrode.

The measurements of the potentials were made with the 3.5 molar KCl-calomel electrodes connected with the tissue studied by means of rubber tubes containing agar-KCl or a string soaked in KCl. The ends of the electrodes introduced into the stomach and Pavlov pouch were prevented from making actual contact with the mucosa by an interrupted barrier of

rubber projections. Measurements of the skin-stomach potential and skin-pouch potential were made by completing the circuit in turn between the stomach or pouch with a third electrode applied to an area of freshly abraded skin (suggestion of Dr. von Kramer). For a few experiments a circuit was made between the skin and an electrode placed in the mouth, the lower esophagus or in the rectum. The stomach-pouch potential was also determined in some experiments. The stomach-pouch potential was always equal to the algebraic difference between the skin-stomach potential and the skin-pouch potential.

After the application of the electrodes, control determinations of the potentials were made for about 30 minutes. The experimental solutions were then introduced and the determinations were continued. Test solutions in quantities of 20 cc. were introduced into the pouch and 50 to 100 cc. into the stomach. In some experiments the solution was introduced at a rate of 1 cc. per minute into the pouch.

RESULTS. 1. *Control measurements.* Preliminary trials showed that the readings during the control period varied with the time interval after the last meal; an observation which is in agreement with that reported by Adair and Goodman. We therefore employed animals fasting for 18 to 22 hours in each experiment.

In experiments made after a fixed fasting period, the potential remained fairly constant, usually within a range of 5 millivolts during a period of 30 minutes. This constancy indicates that the potentials are independent of hunger contractions which are intermittent in character.

The potentials in the stomach and pouch were negative to the skin. The potential of the pouch was approximately 10 millivolts more positive than the stomach. This may be related to the fact that readings for the stomach were taken from the junction of the body of the stomach with the pyloric antrum, whereas the pouch was formed from the body of the stomach.

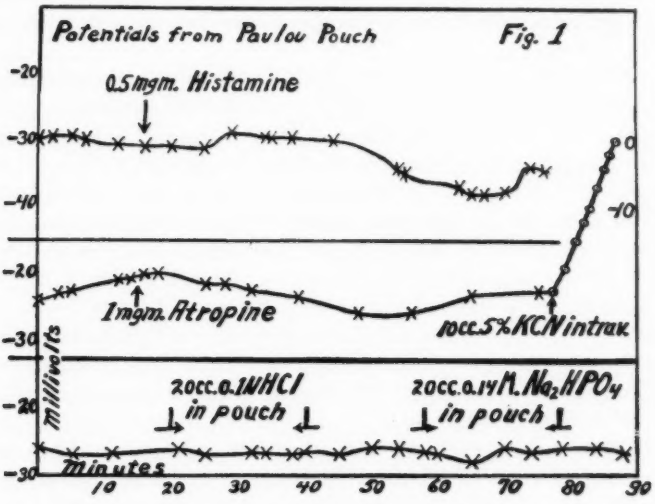
The stomach and pouch potentials were not altered by respiratory or other somatic movements or nervous states of repose or restlessness.

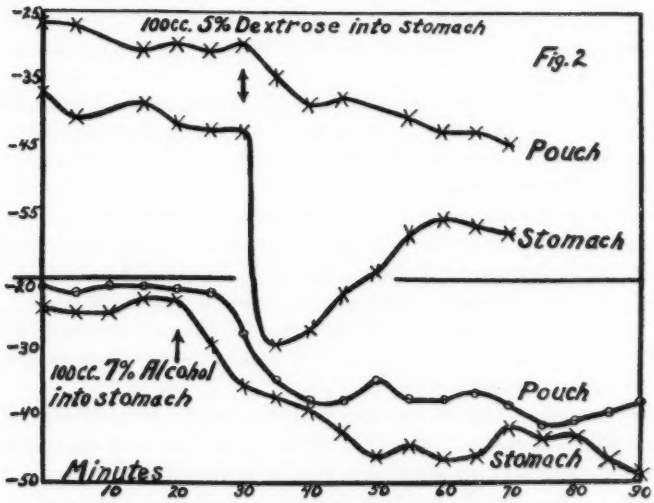
Potential readings were also taken from the mouth and esophagus, which were from 3 to 5 millivolts more negative than the skin. The rectum was also negative (25 millivolts). These values were unaffected by the experimental procedures studied.

2. *Gastric acidity.* No significant changes in the potential were observed when the pouch was perfused with liquids of different acidity, including 0.1 molar HCl (pH 1.08), 0.01 molar HCl (pH 2.04), neutral salt solutions, or 0.14 molar Na_2HPO_4 (pH 9). The typical curve shown in figure 1 shows a range of variation of about 3 millivolts, in changing the pH from 1.08 to 9.

3. *Effects of the injection of histamine, pilocarpine and atropine.* Sarre

observed an increase in gastric acidity and in potential on the injection of histamine. In our experiments, no significant changes in potential were observed during an interval of about 30 minutes after the subcutaneous in-





jection of 0.5 mgm. of histamine. The curve in figure 1 shows an alteration after 30 minutes, but it is not safe to assume that this change is due to the augmentation in gastric secretion for this activity is greatly increased

during the 15 to 30 minute interval following histamine administration and furthermore, the change in potential was not constant in occurrence. Injections of 2 mgm. of pilocarpine or of 1 mgm. of atropine (fig. 1) failed to cause any significant changes in the gastric potentials, although these substances are known to alter secretory and motor activity of the stomach and vagus influence.

The introduction of 7 per cent alcohol into the pouch made the pouch more negative and the presence of alcohol of the same concentration in the stomach increased the negativity of both the stomach and the pouch (fig. 2). The augmentation in gastric secretion produced by these procedures appeared to be unrelated to the changes in potentials. Alcohol administered by any channel augments gastric secretion (ref. Carlson,) but the intravenous injection of 100 cc. 15 per cent alcohol in 5 per cent dextrose solution did not alter the potential of the stomach or pouch.

4. *Hypertonic, isotonic and hypotonic solutions.* No significant changes in the potential were observed when solutions of different salt concentration were introduced into the stomach or pouch. The solutions investigated included distilled water, 0.9 per cent NaCl, 2 per cent NaCl, 2 per cent KCl and 2 per cent urea.

5. *Dextrose.* The potentials became more negative when the pouch was perfused with 5 per cent dextrose. Both pouch and stomach were affected when dextrose was placed in the stomach as shown in figure 2. Contact between the lumen surface of the mucosa with dextrose was essential, for no change in potential followed the intravenous injection of 25 cc. of 50 per cent dextrose.

6. *Milk and related substances.* The introduction of whole milk into the pouch caused the pouch to become more negative, likewise, milk in the stomach lowered the electrical state of the stomach and pouch. The variable results in potential of the stomach reported by Adair and Goodman to follow the drinking of milk were not obtained.

Soluble casein (50 cc. of a 2 per cent solution) introduced into the stomach failed to modify the electrical state of stomach or pouch. The whites of 2 eggs similarly administered was likewise ineffective. Thus casein is shown not to be the essential factor in milk and the type of gastric activity associated with the digestion of casein or egg white does not involve a change in gastric potential.

The action of emulsified fat was investigated by introducing into the stomach 30 cc. of cream or the yolk of two eggs. In each case a decrease in stomach potential occurred but values of the pouch were practically unaltered. These results suggest that fat in milk may play a rôle in the alteration of gastric potential induced by milk. The action of milk, however is not entirely dependent on its fat content, for fat in the stomach does not alter the potential of the pouch as milk does.

Fat administered by the method employed in these experiments inhibits both stomach and pouch motility (Quigley et al.,) and secretion (Lim et al.) through the intermediation of enterogastrone. The ineffectiveness of fat administered intragastrically on pouch potential indicates that enterogastrone is not the factor through which fat modifies gastric potential.

7. Vital activity of the organism is essential for the production of the gastric potential as was demonstrated by its rapid disappearance following the intravenous injection of potassium cyanide (fig. 1).

CONCLUSIONS

The electrical state within the lumen of the stomach or a gastric Pavlov pouch is lower than the abraded skin and during fasting periods is relatively constant in magnitude. The potentials of the two portions of gastric tissue usually differ in magnitude, in time and in extent of change but generally change in the same direction. Our experimental results indicate that gastric potential is not dependent on the pH of gastric contents, or upon the well known types of gastric activity; secretion of acid, pepsin or mucin, on hunger contractions, digestive contractions or other types of motor activity, or upon the diffusion of ions or non-electrolytes. The electrical potential is lowered by contact of milk, emulsified fats, alcohol or dextrose with the gastric mucosal surface, but in the case of the latter two, not by their introduction into the blood stream.

Gastric potential production is dependent on an undetermined type of vital activity, apparently of the gastric mucosa. When altered by contact of the mucosa with effective substances, the influence may be transmitted to gastric tissue not in contact with the excitant.

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THE PHYSIOLOGY OF ZINC IN THE NUTRITION OF THE RAT[1]

E. HOVE, C. A. ELVEHJEM AND E. B. HART

From the Department of Agricultural Chemistry, University of Wisconsin, Madison

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The indispensability of zinc in the nutrition of the rat has been demonstrated in two previous papers from this laboratory (Todd, Elvehjem and Hart, 1; Stirn, Elvehjem and Hart, 2). These results substantiated the observations of Bertrand and co-workers (3, 4) who used mice, and also the work of Hubbell and Mendel (5). Newell and McCollum (6) were unable to show a difference in the growth rates between rats on their purified ration, which contained 1 milligram of zinc per kilo according to spectrographic analysis, and rats on the same ration supplemented with zinc salts. They did find that the young born from rats on their zinc low ration contained much less zinc than those from mothers on the stock ration. Their failure to demonstrate a zinc deficiency has been attributed by Stirn, Elvehjem and Hart to inadequate purification of their ration. Stirn and associates also found a marked decrease in the efficiency of the zinc deficient rats for converting food to body weight.

Scott and Fisher have reported (7) that zinc is chemically bound in the crystalline insulin molecule, and (8) that the injection of zinc salts together with crystalline insulin produced a far less drastic hypoglycemia than insulin alone. The action of zinc was not specific however, for nickel or cadmium could replace zinc in the crystalline insulin molecule with no loss in physiological activity, and nickel salts had the same action as zinc salts when injected with insulin. It has been reported (9) that the pancreas of all animals has a high zinc content, but Fisher and Scott (10) found no correlation between the total amount of zinc in beef pancreas and the insulin content.

Hisaw, Fevold, and Greep (11) have shown that the addition of zinc salts to hypophyseal sex hormone preparations results in a marked augmentation of the response. Iron and copper salts had the same action. They attribute this effect of heavy metals to a colloidal action such as to

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cause the slower and hence more effective absorption of the hormones. It has been reported (12) that gonadectomy of female silkworms brings about a four-fold increase in the zinc concentration of the blood. Gonadectomy of male silkworms had no effect on the zinc content of the blood.

In view of these postulated interrelations between zinc salts and insulin and the pituitary sex hormones, investigations have been made on the relation of zinc to certain aspects of carbohydrate and protein metabolism of the zinc deficient rat, and the effect of whole pituitary and the pituitary growth hormone when injected into the zinc low rats.

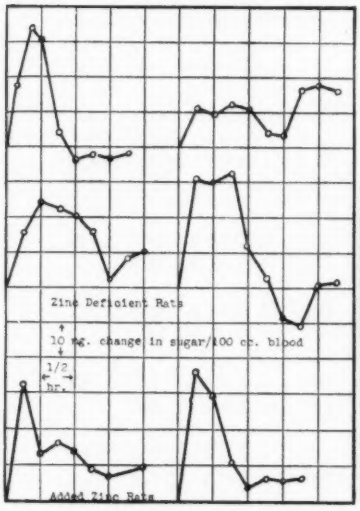

Fig. 1. Glucose tolerance curves

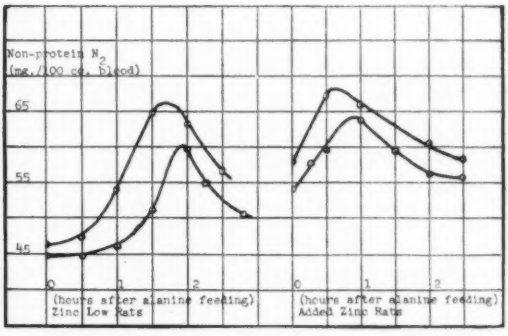

Fig. 2. Alanine "tolerance" curves

Experimental animals. The method of preparing the animals and the zinc low ration is the same as outlined by Stirn et al. (2). It was found that better growth was obtained by the addition of 2 micrograms of vitamin B_1 daily (Sankyo Co., Ltd.) concentrate. The average daily growth over a six-week period using summer milk serum was less than 1.5 gram for rats on the zinc low ration and over 3.0 grams for litter mates on the same ration supplemented with 300 micrograms of zinc as zinc chloride per day. The efficiency of the zinc low rats in converting food eaten to body weight decreased to about 40 per cent of the control value. The average daily zinc ingestion of the zinc deficient rats was 22 micrograms. Increasing the zinc content of the ration to 30 micrograms per day markedly decreased the zinc deficiency and it was found that 40 micrograms of zinc per day entirely prohibited the production of a zinc deficiency.

Carbohydrate and protein metabolism. Glucose tolerance determinations were made by feeding, by pipette, 0.2 gram of glucose per 100 grams of body weight in a 40 per cent solution after a five-hour starvation period. Blood glucose was determined by the Benedict method (13) before the glucose feeding and at quarter-hour intervals thereafter. As is shown in figure 1 the tolerance curves for the zinc deficient rats differ from the normal in some cases. Only a few of the typical curves are given.

The blood sugar of the zinc deficient rats was normal, and they excreted no sugar in their urine as determined by Benedict's urine-sugar method (13). No differences were found in the liver glycogen; determinations were made on three zinc low and two control rats by the usual procedure of dissolving 1 gram or less of the liver in 40 per cent sodium hydroxide, precipitating with alcohol, washing, hydrolyzing with dilute sulfuric acid and estimating the glucose formed. The rats had been starved for 24 hours prior to killing by decapitation.

TABLE 1

Protein metabolism studies

ANALYSIS FOR:	GROUP	NUMBER OF RATS	AVERAGE		RANGE	AVERAGE VALUE	PER CENT DIFFERENCE
			Age	Weight			
			weeks	*grams*			
Blood non-protein N_2 (mgm./100 cc.)	−Zn	8	6	110	41.0–52.0	46.3	−14
	+Zn	7	6	170	48.0–58.0	53.8	
Rise of blood N_2 after alanine feeding (mgm./100 cc.)	−Zn	6	6	105	13.0–20.0	16.0	+45
	+Zn	4	6	160	10.0–12.0	11.0	
Serum protein, I (gms./100 cc.)	−Zn	3	4	100	5.4–5.7	5.5	−14
	+Zn	3	4	140	6.3–6.4	6.4	
Serum protein, II (gms./100cc.)	−Zn	2	4	110	6.2–6.3	6.3	0
	+Zn	2	4	150	6.3–6.4	6.4	
Urinary creatinine (mgm./24 hours)	−Zn	3	8	105	2.73–3.68	3.18	−20
	+Zn	2	8	145	3.94–4.00	3.97	

To determine whether the irregularities of the glucose tolerance curves could be due to delayed absorption from the intestinal tract the blood non-protein nitrogen level was followed after alanine feeding. After a five-hour starvation period 0.2 gram of d-l alanine per 100 grams of body weight was fed in solution by pipette. The non-protein nitrogen was determined before the alanine feeding and at half-hour intervals thereafter by the Wong modification of the Folin and Denis method (13). Figure 2 shows a

few of the typical curves. In all cases the zinc deficient rats displayed a distinct delay in the time required for the blood nitrogen to reach a maximum. The initial non-protein nitrogen level of the zinc deficient rats was lower and the total rise above this basal level on alanine feeding was

TABLE 2

Urinary and fecal nitrogen

	ZINC LOW RATS	ADDED ZINC RATS	PER CENT DIFFERENCE
Number of rats	3	2	
Average of body weight (gm.)	120.0	170.0	
Average food/day (gm.)	3.74	5.55	
Urinary N_2/day (mgm.)	43.0	68.0	−36.8
Urinary N_2/gm. food	11.5	11.6	0
Feces weight (dry)/day (gm.)	0.281	0.242	+16.0
Fecal N_2/day (mgm.)	17.2	15.7	+9.4
Fecal N_2/gm. food (mgm.)	4.66	2.70	+72.6
Fecal N_2/gm. feces (mgm.)	60.1	58.2	+3.3

TABLE 3

Growth response to whole pituitary

RAT		AGE	INITIAL WEIGHTS	WEIGHT CHANGE			FOOD	EFFICIENCY $\left(\frac{\text{FOOD}}{\text{WT.}}\right)$
		weeks	*grams*	*grams/week*	*per cent*	*average*	*grams/week*	
Injected	150	3	74	10	13.5	21.3%	32	3.2
	152	3	63	22	35.0		47	2.1
	180	8	133	19	14.0		33	1.8
	182	8	127	14	11.0		27	1.8
	70	6	70	26	37.0		69	2.7
	71	6	100	14	14.0		58	4.1
	72	6	95	23	24.0		67	2.9
Control	70	5	66	4	6.0	7.1%	25	6.1
	71	5	94	6	6.0		27	4.5
	772	5	89	8	7.7		26	4.4
	151	3	68	8	12.0		28	3.5
	181	8	127	5	4.0		15	3.0

greater (see table 1). Serum protein was determined by the method of Greenberg (14) and urinary creatinine by the micro method of Folin (13). These results are shown in table 1.

Urinary and fecal nitrogen analyses were made by the usual Kjeldahl method. Twenty-four hour urine samples were collected by placing the

cages over large glass funnels which fitted tightly into large test tubes. A glass ball was placed in the neck of the funnel to catch the feces and prevent evaporation of the urine. The feces were dried at 50°C. for 24 hours. The results are given in table 2.

Zinc and pituitary effects. Whole pituitary transplants to zinc deficient rats were conducted as follows: The gland was aseptically removed from stock rats, tamped into a sterilized spinal needle and forced deep into the muscle of the hind leg of the rat with the plunger. One whole pituitary was injected daily for one week. Weights were recorded every other day. Food consumption records were kept and the efficiencies of converting food to body weight (as the ratio of the weight of the food eaten to the increase in body weight) calculated. The results are shown in table 3. The rat pituitary could not be analyzed for zinc because of its small size, averaging only about 25 mgm.; but fresh beef pituitary showed a zinc content of 15.0 mgm. zinc per kilo and fresh hog pituitary contained 12.8 mgm. per

TABLE 4

Growth response to antuitrin-G

	RAT	AGE	INITIAL WEIGHTS	WEIGHT CHANGE		
		weeks	*grams*	*grams/ week*	*per cent*	*average*
Injected	62	15	140	10	7.2	7.4%
	61	15	142	9	6.5	
	50	15	128	11	8.5	
Control	51	15	132	5	3.8	3.2%
	60	15	130	3	2.3	

kilo. On the assumption that the rat pituitary has the same order of zinc concentration as the beef gland, each rat pituitary would contain 0.3 microgram of zinc.

"Antuitrin-G" growth hormone (a commercial preparation) was injected intraperitoneally into zinc deficient rats at a level of 1.5 rat unit per day for one week. The results are shown in table 4. One cubic centimeter of the Antuitrin-G is equivalent to 10 rat units and contains 30 micrograms of zinc. The method of Todd and Elvehjem (15) was used for all zinc analyses.

DISCUSSION. A number of facts strongly indicate that the zinc deficiency in rats is accompanied by a decreased absorption from the intestinal tract.

There is usually a distinct irregularity in the glucose tolerance curves of the zinc low rats. In view of the fact that there is no sugar lost in the urine and that blood sugar and liver glycogen were normal this disturbance

in glucose tolerance can better be explained as due to a delay in the absorption rate from the intestines rather than to any deeper seated carbohydrate disorder.

The decrease in efficiency of converting food to body weight can be explained by this finding. It is interesting that one group of rats on the zinc low ration developed pellagra-like symptoms (bespectacled eyes, lesions in the mouth corners and on the feet) although the added zinc controls did not. The same amount of liver extract and milk serum had been consumed each day by both groups. When the liver extract was increased from 0.05 to 0.1 gram per day the symptoms cleared up.

The delayed absorption hypothesis is further strengthened by the fact that in the zinc deficient rats there is a distinct increase in the time required to reach the maximum blood non-protein nitrogen level after alanine feeding. It is interesting that one rat showed a very normal glucose tolerance curve but the alanine tolerance of the same rat is typically abnormal. In general, the disturbance in the glucose tolerance is much less than in alanine tolerance. This may indicate a preferential delay in nitrogen absorption.

Another indication of delayed absorption is the fact that the weight of the dry feces excreted per day by zinc low rats is greater than for controls, even though the zinc low rats consume less food. Correspondingly, the fecal nitrogen per gram of food eaten is greater for the zinc deficient animals. The nitrogen per gram of dry feces is the same for both groups.

The total serum protein of one litter of zinc low rats was 14 per cent lower than the normals. Another litter with apparently as great a deficiency showed no lowering of blood protein. Bloomfield (16) has reported that rats kept on a complete ration but with less than 18 per cent protein in their diet show a 13 per cent drop in serum protein; if the dietary protein is over 18 per cent the serum protein is normal. The zinc low ration contained 19 per cent of protein, but assuming a preferential delay in the absorption of nitrogenous products the effective percentage of protein may have been low enough in some cases to produce the Bloomfield effect.

The total nitrogen excreted in the urine per gram of food eaten is about the same for both groups. The urinary creatinine is 20 per cent low. These two facts can not be explained by delayed absorption alone, for it would be expected that with a relative decrease in the amount of nitrogenous products absorbed into the blood stream there would be a corresponding fall in the urinary nitrogen per gram of food eaten. A slower endogenous metabolic rate in the zinc deficient rats would result in a less efficient utilization of the absorbed nitrogenous products and a corresponding increase in excreted nitrogen as well as a fall in urinary creatinine.

Whole pituitary gland transplants gave a definite growth response in the zinc low rats. It is highly improbable that the extra 0.3 microgram of

zinc obtained by the rat per day in this technique could produce any of the effect noticed, even though injected material is more available than oral administration as has been shown in the case of copper (17). The efficiency in converting food to body weight in the injected animals showed a sharp increase, approaching normal values in some cases. The positive effect of the pituitary on growth and food utilization would hardly be expected unless there was some connection between the zinc deficiency and one or more of the hormones of the pituitary.

The injection of the pituitary "growth" hormone (Antuitrin-G) at a level of 1.5 rat unit per day also gave a response. A rat unit is defined as the amount of hormone needed to give an increase in body weight of 10 per cent per day or 70 per cent per week in plateaued female rats. The observed response amounted to only 7.4 per cent per week. This is about one-third of the growth response given by the whole pituitary transplants. It is possible that the entire effect of the growth hormone (Antuitrin-G) is due to the additional 4.5 micrograms of zinc per day obtained by the rat in this procedure since it has been shown that the zinc deficiency is decreased in severity by adding 6 micrograms of zinc per day to the ration, and is prevented entirely by an increase of 18 micrograms per day.

It may be postulated on the basis of this evidence that zinc is involved in the production or utilization of some hormone of the pituitary gland which controls the motility and tonus of the intestinal tract, the lack of which causes delayed absorption and slower growth. It is possible that with a more severe zinc deficiency other and more basic metabolic disturbances will become evident.

SUMMARY

1. A zinc deficiency in rats is prevented when the animals receive 40 micrograms of zinc per day. The zinc deficient rats used for metabolic studies were receiving 22 micrograms of zinc per day and showed a 50 per cent decrease in growth rate.

2. No disorders in the carbohydrate metabolism of these rats could be detected. Urinary sugar, blood sugar, liver glycogen and glucose tolerance were determined.

3. A number of facts have led to the conclusion that there is a marked delay in intestinal absorption in the zinc deficient rat and that this delay is probably greater for nitrogenous products than for carbohydrates.

4. It is suggested that zinc deficient rats have a lower rate of endogenous metabolism. This would explain the fall in creatinine and the maintenance of a normal urinary nitrogen excretion per gram of food eaten in spite of the demonstrated delayed absorption.

5. Whole pituitary transplants to zinc deficient rats produced a marked growth stimulation and an increase in efficiency of converting food to body

weight. It was shown by Antuitrine-G "growth" hormone injections that this effect could not be due to the zinc content of the pituitary gland nor to its "growth" hormone content.

6. It is postulated that zinc is involved in the production or utilization of some hormone of the pituitary which controls the motility and tonus of the intestinal tract. More drastic zinc deficiencies than have as yet been produced may disclose more basic metabolic disturbances.

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A MECHANISM OF LYMPHATIC ABSORPTION FROM SEROUS CAVITIES

LANE ALLEN AND ELKIN VOGT

From the Department of Anatomy, and the Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta, Georgia

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Although a voluminous literature has developed on the subject of lymphatic absorption from serous cavities, the exact mechanism of the process remains obscure. Peritoneal lymphatic absorption is generally considered to be the result of intra-abdominal pressure, the rubbing effect of viscera and the vital activity of peritoneal mesothelium.

Our interest in the problem grew out of recent histological studies (Allen, 1) in which it was noted that the subdiaphragmatic subserous lymphatic lacunae were compressed and their lumina obliterated during contraction of the diaphragm. This observation caused us to doubt the generally accepted idea that materials are forced through the peritoneum and into the lymphatic lumen by intra-abdominal pressure, since in the contracted diaphragm the lymphatic lumen is not patent.

The present investigation, which consists of direct observations on lymphatic absorption and flow, demonstrates a mechanism of absorption common to the major serous cavities of vertebrates and which is possibly operative for the lymphatics of tissues in general.

EXPERIMENTS AND RESULTS. If diaphragmatic absorption depends upon intra-abdominal factors such absorption should not occur in animals with the abdomen open and the liver retracted from the diaphragm. The following experiments demonstrate that intra-abdominal factors are not necessary for lymphatic absorption in the rabbit.

METHOD. Rabbits were anesthetized with paraldehyde, 1.7 cc. per kilo., placed in the dorsal postion, and the abdomen opened by midline incision. The efferent diaphragmatic lymphatics were exposed in the first intercostal space (2). The liver was retracted and 10 cc. of Hydrokollag 300 (3) were applied upon the diaphragm. Respiratory movements and the time interval between injection and the appearance of graphite in the first interspace were recorded. The peritoneal cavity was then washed with warm Ringer solution and sponged with cotton. Time was allowed for clearing of graphite from the lymphatics of the first interspace. A second injection was then made, the liver replaced and the abdominal incision tightly closed.

This method was used on twelve rabbits in one of which, no. 4, six injections were made at regular intervals over a period of approximately two hours. This procedure afforded opportunity to determine the volume output of diaphragmatic lymphatics under different respiratory conditons. Since it is impractical to cannulate the numerous small efferent lymphatics, indirect measures were necessary. Lymphatic flow of graphite suspension was calculated from the observed rate of flow for each injection and the average cross-sectional area of the injected efferent lymphatics, determined under the microscope.

The point to be stressed in a consideration of table 1 is not that lymphatic absorption is correlated with movements of the diaphragm, there being general agreement here (4, 5, 6, 7) but that intra-abdominal factors are not necessary for lymphatic absorption from the peritoneal cavity of the rabbit. A comparison of injections 2 and 5 shows that with stronger respiratory movements lymphatic absorption may be more rapid with the diaphragm exposed than with abdominal viscera in situ.

TABLE 1

Rabbit 4. Wt., 2.25 kgm.; 5.2 cc. paraldehyde. During injections 1, 3, 5 and 6 abdomen was open, liver retracted. During injections 2 and 4 abdomen was closed, liver in situ. Preceding injection 6, 4 cc. paraldehyde were injected into loop of ileum

INJECTION NUMBER	AMPLITUDE OF RESPIRATION	RESPIRATORY RATE PER MINUTE	TIME FOR GRAPHITE TO APPEAR IN 1ST INTERSPACE	RESPIRATORY EXCURSIONS OF DIAPHRAGM	CALCULATED FLOW PER HOUR
			seconds		*cc.*
1	Moderate	49	167	136	2.9
2	Increasing	49	112	91	4.4
3	Increasing	53	93	82	5.3
4	Increasing	52	78	67	6.6
5	Increasing	52	63	55	7.8
6	Shallow	42	243	170	2.0

Lymphatic absorption by the exposed diaphragm of the dog, which animal has been used by the majority of investigators, is a very ineffective process. The following experiments demonstrate, however, that we are not dealing with different mechanisms of absorption in the rabbit and dog, but with modifications of the same fundamental mechanism.

Absorption by excised diaphragms and other tissues.[1] The central tendon of the diaphragm of the rabbit is composed of pleural and peritoneal strata of parallel tendon fibers, the fibers of one stratum crossing obliquely

[1] Experiments upon lymphatics suitable for student laboratory exercises are few. Experiments upon excised tissues and upon the living rabbit with diaphragm exposed have been used in this laboratory to demonstrate the mechanism and sites of absorption of serous fluid, blood, bacteria, etc., from serous cavities.

the fibers of the other stratum. Between the peritoneal tendon fibers lie numerous subserous lymphatic lacunae which communicate with a deeper plexus lying beneath fibers of the pleural stratum. When a drop of India ink was placed upon the peritoneal surface of the excised central tendon, a single stretching usually sufficed to completely fill the plexus with ink. Subsequent stretching and manipulation resulted in a flow of the absorbed ink into and along the efferent pleural lymphatic plexus. This absorption and flow could be effected not only in the fresh diaphragm but in one which had been fixed and preserved for several days. Under the microscope it was noticed that stretching in the direction at right angle to the length of the subperitoneal tendon fascicles tended to separate these fascicles and greatly increased the volume of the lymphatic plexus, while stretching parallel to the peritoneal tendon fascicles compressed the plexus and forced the absorbed material along the efferent channels. The alternate changes in lymphatic volume which result in absorption and flow are illustrated in figure 1.

The sub-diaphragmatic lymphatic plexus in the dog has been described by MacCallum (4) as consisting of subserous lymphatic terminals or lacunae which communicate with deeper channels which parallel the muscle fascicles. We injected this plexus by compressing the excised diaphragm with the fingers and applying a drop of ink at the site of compression. As the tissue expanded the ink was absorbed through the peritoneum, outlined the lacunae, coursed through the communicating channels and filled the deep lymphatic channels which parallel the muscle bundles (fig. 2, A). Absorbed ink even coursed along the deep lymphatics for a short distance peripheral to the margin of the drop of ink. When the diaphragm was now stretched the subserous lymphatic plexus increased still more in volume and a heavier injection resulted (fig. 2, B). Subsequent stretching and relaxing of the diaphragm failed to result in flow of the absorbed ink. However, when the pressure of the liver was simulated by rubbing the peritoneum with the finger, the absorbed ink passed through the diaphragm and appeared in the efferent pleural plexus.

Lymphatic absorption per se, in the dog and rabbit, thus depends upon the same mechanism, i.e., an increase in volume of lymphatic terminals coincident with relaxation and stretching of the diaphragm. Lymphatic flow and coincident evacuation of the subserous lymphatic plexus is, in both animals, the result of compression of the lymphatic plexus during contraction of the diaphragm. In the rabbit this compression is the result of changes in the pattern of the central tendon fiber arrangement. In the dog compression of the plexus depends upon the pressure of the liver against the contracting diaphragm. Hence in the dog retraction of the liver interferes primarily with the mechanism of lymphatic flow.

The subserous lacunae of the mouse represent the simplest form of ab-

sorptive apparatus and are illustrated in figure 3. All drawings were constructed from histological sections and from direct observations on absorption under the dissecting microscope.

Von Recklinghausen (8) placed ink, milk, cinnabar, etc., on the excised diaphragm and under the dissecting microscope observed vortices in the suspensions in the region of the so-called stomata, and a flowing of the absorbed material along subserous lymphatics. MacCallum (4) was un-

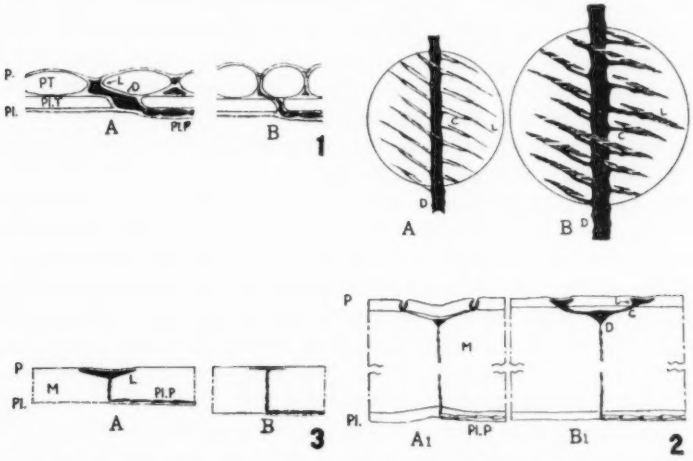

Fig. 1. Oblique cross-section of central tendon of rabbit diaphragm showing volume changes in lymphatic plexus. *A*, diaphragm relaxed; *B*, diaphragm contracted.

Fig. 2. Diaphragmatic lymphatic plexus of dog. *A*, *A1* show filling of plexus with ink during expansion of pressed diaphragm; *B*, *B1* show further absorption with stretching. *A*, *B*, surface view of diaphragmatic peritoneum. *A1*, *B1*, cross-section of diaphragm. Circles represent periphery of drop of ink.

Fig. 3. Cross section of diaphragm of mouse, illustrating simplest form of subserous lymphatic plexus. *A*, diaphragm relaxed; *B*, diaphragm contracted against liver. *C*, communicating lymphatic; *D*, deep collecting lymphatic; *L*, subserous lymphatic lacuna; *M*, musculature of diaphragm; *P*, peritoneum; *Pl*, pleura; *Pl. P.* pleural efferent plexus. *PT*, tendon fiber of peritoneal stratum; *Pl.T.*, tendon fiber of pleural stratum.

able to confirm this finding. It is evident that our demonstration of the first phase of lymphatic absorption from the excised diaphragm of the dog is a repetition of Von Recklinghausen's experiment. The subserous lymphatics must be evacuated before placing the suspension upon the diaphragm and the tissue must be in such a state that it can expand freely. Under the dissecting microscope we observed vortices in the suspension at points which correspond to the depressed roofs of the lymphatic lacunae

(fig. 2, L). What Von Recklinghausen mistook for patent communications and called stomata are without doubt the depressed "roofs" of the lymphatic lacunae.

The mechanism of pleural lymphatic absorption is similar to diaphragmatic absorption. The extensive intercostal sub-pleural lymphatic plexus was injected in a few seconds by pouring ink upon the pleura of the excised thoracic wall and repeatedly stretching the intercostal spaces.

The pericardial lymphatics were found to be injected within 2 to 5 minutes after intra-pericardial injecting of ink or graphite suspension. Observations on the injected pleural and pericardial lymphatics, under the dissecting microscope, showed the structural modifications characteristic of the diaphragmatic lymphatic mechanism.

Inasmuch as many lower forms lack both the functional diaphragm and ribs of mammals it was felt that the study of absorption from the pleuroperitoneal cavity might further elucidate the problem. Ink was injected into the pleuroperitoneal cavities of the turtle and frog and the abdomen was opened within five to ten minutes and the excess ink washed out. In the turtle an extensive subserous lymphatic plexus was found filled with absorbed ink. This plexus lies deep to the dorsal peritoneum which is reflected over the lungs, the lungs being retroperitoneal. As the lungs fill with air, the dorsal peritoneum is stretched away from the body wall in such a way that the lymphatic volume increases and "sucks" the suspension into the lymphatic plexus. During collapse of the lung the plexus is compressed and the suspension is forced along the efferent channels.

Lymphatic absorption in the frog takes place through the dorsal peritoneum into a large lymphatic space, the cisterna magna. As the abdominal wall relaxes during inspiration the dorsal peritoneum is stretched, the volume of the cisterna magna is increased and the suspension is "sucked" through the stretched dorsal peritoneum. The evacuation of the cisterna in the frog is possibly a function of the lymph hearts.

The subserous lymphatics of the frogs and turtles were likewise made to absorb by simulating the normal movements of the tissues.

Discussion. In considering the mechanism of lymphatic absorption it is necessary to make a distinction between the filling of the lymphatic terminals, which is lymphatic absorption per se, and the flow of absorbed materials. Absorption per se is due to an increase in volume of lymphatic terminals. In some instances this is the result of lifting of the depressed roofs of the lymphatic lacunae (fig. 3). In another instance this increase in volume is due largely to expansion and stretching of the deep collecting lymphatic channels (fig. 2).

It should be stressed that lymphatic absorption per se from serous cavities does not depend upon absolute pressure in the cavity nor upon the

pressure of viscera. Such absorption depends upon a difference in pressure on the two sides of the sero-endothelial membrane which separates the lymphatic lumen from the serous cavity, this difference in pressure being due to a change in volume of the subserous lymphatic plexus. The structure of this membrane which separates the peritoneal cavity from the lymphatic lumen has been studied extensively (1, 4, 9, 10). Allen (1) has demonstrated the passage of particles through intercellular openings and has redefined stomata as openings in the peritoneum overlying lymphatics, through which the peritoneal cavity comes into direct contact with the intercellular cement of the lymphatic endothelium. At certain points, then, the only barrier between the serous cavity and the lymphatic lumen is the intercellular cement. It was concluded that the lymphatic intercellular cement was rarefied in the regions of the stomata as the membrane was stretched and that definite openings occurred through which particles and solutions were "sucked" by the coincident pressure differential. The almost instant filling of the lymphatic plexus with a suspension of particles by a single stretching of the tissue will admit of no other explanation than that the lymphatic endothelium and overlying serous membrane are physiologically and anatomically sieve-like during the process of absorption. During absorption retrograde flow into lymphatic terminals is prevented by the lymphatic valves.

The lymphatic pumps, in the instances which we have described, are confined to regions of extensive motion and are operated by respiratory movements. We have been unable to demonstrate lymphatic absorption by manipulation of the peritoneum of the body wall, mesentery, viscera, etc. It is quite possible that lymphatic pumps may be operative in other serous cavities. A characteristic fenestrated membrane, suggestive of this mechanism, has been described for the tunica vaginalis testis and it has been shown that active and passive movements effect lymphatic absorption from joint cavities.

There is no direct evidence that the principles of lymphatic absorption which we have described are applicable to lymphatic absorption from the tissue spaces of the body in general. However, the ease with which lymphatics in all locations are entered by solutions and suspensions and the dependence of lymphatic absorption upon passive and active movements of tissues suggest the identity of the processes.

SUMMARY

1. Lymphatic absorption from the major serous cavities of vertebrates has been studied by means of direct observations on absorption of suspensions. A method is described whereby diaphragmatic lymphatics can be alternately injected and flushed by particulate matter, permitting the

study of absorption under different experimental conditions. A method is described also whereby absorption can be observed and studied in excised preparations.

2. The filling of terminal subserous lymphatics is coincident with expansion and stretching of tissues in which lymphatics are situated and lymphatic flow is the result of compression of lymphatics during the contraction or compression of surrounding tissue. Lymphatic absorption is a discontinuance process and a sharp distinction should be made between absorption and flow.

3. An increase in volume of lymphatic terminals is coincident with relaxation of subserous tissues. This results in a difference in pressure on either side of the sero-endothelial membrane which separates the lymphatic lumen from the peritoneal cavity and particles and solutions are "sucked" into the lymphatic lumen. The sero-endothelial membrane is physiologically sieve-like and the penetration of the membrane and the filling of the lymphatic lumen is an almost instantaneous process.

4. The mechanism of lymphatic compression and flow presents modifications in different locations. The contraction of the diaphragm against the liver is necessary in those animals whose lymphatics are confined to the muscular portion of the diaphragm, such as the dog, man, and mouse. In the rabbit, pressure of viscera is not necessary, the lymphatics being so arranged within the central tendon that contraction alone will compress the plexus.

5. The principles of absorption described for lymphatics of the diaphragm are shown to apply to other serous cavities, pleural, pericardial, and pleuro-peritoneal cavities of lower forms.

6. Lymphatic absorption from serous cavities and lymphatic absorption from the tissues in general present many similar phenomena and it is suggested that the mechanism described here may be operative for the lymphatics of tissues in general.

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THE INFLUENCE OF A PANCREAS EXTRACT ("FAT METABOLIZING HORMONE") UPON FAT DEPOSITION IN THE LIVER ON A LOW PROTEIN DIET

EATON M. MacKAY

From The Scripps Metabolic Clinic, La Jolla, California

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Dragstedt and his co-workers (1) have prepared an alcoholic pancreas extract, believed by them to contain a fat metabolizing hormone, which permits the survival and prevents the fatty infiltration of the livers of depancreatized dogs. In using this material for experimental work the necessity of having depancreatized dogs as test animals presents obvious difficulties. Experiments were consequently carried out on rats which develop fatty livers on low protein high fat diets (2, 3). The results are of interest in themselves and because they appear to offer an easily used and more uniform test animal for use in fractionation and standardization of this possible hormone from the pancreas.

The low protein diet used for producing infiltration of the liver with fat was essentially the same as the one devised by Channon (4). It consisted of glucose 45, butter fat 40, casein 5, dried powered medicinal brewers' yeast 5 and the usual salt mixture (5) 5. The pancreas extract was prepared according to Dragstedt's directions (1) and used in aqueous solution without added salt in a concentration such that 1 cc. was equal to 10 grams of the original fresh pancreas. This extract contained 20.36 per cent total solids and 2.42 per cent nitrogen. After appropriate periods on the diet and extract the rats were etherized and then bled to death from the large abdominal vessels so that the liver was freed of excess blood. Liver fat was determined by a saponification procedure (6) and therefore represents fatty acids plus unsaponifiable lipid material.

Average results comprise table 1. All of the rats fed on Channon's (4) high fat, low casein diet stored large quantities of fat in their livers. An interesting observation is the consistently higher liver fat concentrations in female rats than male rats (expt. 1, grps. 4 vs. 2; expt. 3, grps. 1 vs. 3, grps. 5 vs. 7, grps. 9 vs. 11). This has been noted before in the lower fat concentrations of rats on ordinary diets by Butts and Deuel (7). In general the younger the rats the larger the amount of fat deposited in their livers. This is particularly well shown in the female rats in experiment 3. For rat groups (1, 5 and 9) weighing an average of 173, 127 and 110 grams the

TABLE 1

GROUP NUMBER AND SEX	NUMBER RATS IN GROUP	INITIAL BODY WEIGHT	BODY SURFACE	BODY WEIGHT WHEN KILLED	LIVER WEIGHT		LIVER FAT		DAYS ON LOW PROTEIN, HIGH FAT DIET	DAYS FASTING*	DAYS "FAT HORMONE" ADMINISTERED*	"FAT HORMONE" PER 100 SQ.CM. B.S. PER DAY	FOOD INTAKE IN GRAMS PER 100 SQ.CM. B.S. PER DAY
					Actual	Per 100 sq.cm. B.S.	Per cent	Per 100 sq.cm. B.S.					
Experiment 1. Fasted after a period on the low protein, high fat diet													
		grams	*sq.cm.*	*grams*	*grams*	*grams*		*mgm.*				*grams*†	*grams*
1♂	4	155	327		5.26	1.61	9.14	147	18	0	0		
2♂	4	155	327		3.91	1.20	4.08	49	18	3	0		
3♂	4	154	326		4.01	1.23	4.31	53	18	3	3	9.2	
4♀	2	118	273		4.94	1.81	12.94	234	18	0	0		
5♀	2	118	273		3.78	1.39	4.71	65	18	3	0		
6♀	2	117	271		3.56	1.31	1.64	22	18	3	3	3.7	
7♀	2	117	271		3.67	1.35	0.93	13	18	3	3	11.1	
8♀	4	231	426	231	7.18	1.69	10.61	179	10	0	0		
9♀	3	246	445	226	6.15	1.38	7.53	104	10	2	0		
10♀	3	208	399	177	5.93	1.48	5.45	81	10	4	0		
11♀	3	242	440	226	6.28	1.43	5.10	73	10	2	2	6.8	
12♀	3	206	396	161	6.28	1.59	3.78	60	10	4	4	7.6	
Experiment 2. Fasted directly from the stock diet													
1♀	2	259	460	259	7.85	1.71	1.53	26	0	0	0		
2♀	3	245	444	203	6.03	1.36	3.32	45	0	4	0		
3♀	3	250	450	214	6.61	1.47	2.59	38	0	4	4	6.7	
Experiment 3. No fasting, kept on the low protein, high fat diet													
1♀	4	173	352	165	6.12	1.74	17.21	299	13	0	0		2.08
2♀	4	173	352	181	7.05	2.00	5.18	104	13	0	13	8.6	2.16
3♂	4	188	373	173	7.67	2.06	10.72	221	13	0	0		2.22
4♂	4	188	373	192	7.29	1.96	5.61	110	13	0	13	8.0	2.11
5♀	2	127	287	110	5.89	2.05	24.60	504	14	0	0		2.23
6♀	2	128	288	111	4.92	1.71	19.93	341	14	0	1	10.4	1.77
7♂	2	170	348	152	5.58	1.61	15.25	246	14	0	0		2.07
8♂	2	168	345	157	5.64	1.64	6.38	105	14	0	1	8.7	1.91
9♀	2	110	260	96	6.80	2.61	31.80	830	16	0	0		2.54
10♀	2	108	257	97	4.86	1.89	24.50	456	16	0	3	11.7	1.99
11♂	2	159	333	143	5.77	1.73	14.85	257	16	0	0		1.98
12♂	2	159	333	149	5.67	1.71	10.43	178	16	0	3	9.0	2.27
Experiment 4. No fasting, kept on the low protein, high fat diet													
1♀	5	137	301	132	5.08	1.69	3.00	54	10	0	10	10.0	2.29
2♀	5	138	302	131	5.14	1.71	11.70	200	10	0	10	2.0	2.20
3♀	5	137	301	130	5.28	1.76	13.20	232	10	0	10	1.0	2.39
4♀	5	138	302	132	5.40	1.79	17.00	305	10	0	10	0.4	2.42
5♀	5	139	307	127	5.78	1.88	21.50	440	10	0	0		2.41

* Period preceding sacrifice for liver analysis.

† Equivalent in grams of fresh pancreas from which extract was prepared.

amount of liver fat per 100 sq. cm. body surface is 299, 504 and 830 mgm. respectively. The food intake which may have some bearing on this varies in the same direction (2.08, 2.23 and 2.54 grams). It is interesting that rats receiving the pancreas extract maintain their body weight better than those which receive none when both are on the diet used here.

The excessive deposition of fat in the liver on the low protein, high fat diet may be prevented by feeding choline (8, 9) or increasing the amount of casein in the diet (9). It is shown here that pancreas extract, prepared according to Dragstedt (1) and presumably containing a new fat metabolizing hormone, when given orally also prevents the deposition of fat in the liver. In experiment 1 when rats with a high liver fat were fasted the amount of fat in the liver became reduced. When the pancreas extract was administered this reduction in liver fat was considerably greater. When rats kept on the fatty liver producing diet (expt. 3, grps. 5–12) were given the liver extract the liver fat also became reduced. Rats which were given the pancreas extract during all of the time (expt. 3, grps. 1–4; expt. 4) they were on the diet which caused the depostion of fat in the liver failed to have as much fat deposited in their livers as those which received no extract.

In experiment 4 an attempt was made to determine approximately the quantitative effect of the pancreas extract. Increasing amounts of extract were less and less effective in relation to their effect on the level of the liver fat. The smoothness of the curve representing the relationship indicates that this method may be very useful for standardization and fractionation of this extract. In our experience a better measure of the strength or lipotropic activity of the extract is through its ability to prevent the accumulation of fat in the liver as in this experiment rather than from its influence on the rate of disappearance of liver fat already deposited.

At present we are uncertain as to the cause of the influence of this pancreas extract upon the fat content of the livers of rats on a low casein, high fat diet but tentatively assume that it is due to the fat metabolizing hormone postulated by Dragstedt et. al. (1). They ruled out the possible influence of lecithin or choline (10) in the effect on depancreatized dogs but these must still be considered here. Our experience with these compounds in relation to this diet make it improbable that the amount of pancreas used could contain sufficient of either to produce the effects we observed. It should be noted that in the dosage used the administration of pancreas extract added a considerable amount of protein derivatives to the diet of each rat. Fractionation of the pancreas extract is now in progress to determine the nature of the material responsible for the effect.

SUMMARY

Rats fed a diet low in protein and high in fat have a large amount (9–32 per cent) of fat deposited in their liver. More fat is deposited in the liver

of females than males. Under the same conditions young rats have a higher liver fat than old rats on this diet.

The oral administration of an extract prepared by alcoholic extraction of fresh pancreas and believed by Dragstedt to contain a fat metabolizing hormone caused the following: A more rapid disappearance of fat stored in the livers on fasting; a reduction in the amount of fat in the liver of rats kept on the low protein fatty liver producing diet; and tended to prevent the infiltration of the liver with fat on this diet according to the amount which was administered.

The use of a low protein high fat diet capable of producing fatty livers in rats is suggested as a method for the standardization and fractionation of pancreas extracts influencing the liver fat.

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$$\frac{20}{1000}$$

$$.2 = 19/100 = 1000\ mg/1\ liter = .2\ M.$$

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